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(54) Title: A NEW ENZYME ISOLATED FROM A *BIFIDOBACTERIUM*

(57) Abstract: The present invention concerns a new β-galactosidase with transgalactosylating activity isolated from Bifidobacterium bifidum and a truncated enzyme where the C-terminal end of the β-galactosidase protein has been deleted resulting in an enzyme with a higher transgalactosylating activity than hydrolase activity. When lactose is used as a substrate, galacto-oligosaccharides are products of the transgalactgosylase activity. Galacto-oligosaccharides enhance growth of health-promoting Bifidobabacterium that may be used in a number of applications in the diary industry.

A new enzyme isolated from a *Bifidobacterium*

Technical field of invention

The present invention concerns improvement of fermented diary products. In particular, the invention concerns a β-galactosidase with transgalactosylating activity. More particular the inventions concerns a β-galactosidase isolated from *Bifidobacterium bifidum* where the C-terminal end of the protein has been deleted and the resulting truncated enzyme has higher transgalactosylating activity than hydrolase activity. When lactose is used as a substrate, galacto-oligosaccharides are products of the transgalactosylase activity. Galacto-oligosaccharides enhance growth of health-promoting *Bifidobacterium* that may be used in a number of applications in the diary industry.

Background of the invention

The genus *Bifidobacterium* is one of the most commonly used types of bacteria cultures in the diary industry for fermenting a variety of diary products. Ingestion of *Bifidobacterium*-containing products furthermore has a health-promoting effect. This effect is not only achieved by a lowered pH of the intestinal contents but also by the ability of *Bifidobacterium* to repopulate the intestinal flora in individuals who have had their intestinal flora disturbed by for example intake of antibiotics. *Bifidobacterium* furthermore has the potential of outcompeting potential harmful intestinal micro-organisms.

Galacto-oligosaccharides are known to enhance the growth of *Bifidobacterium*. This effect is likely achieved through the unique ability of *Bifidobacterium* to exploit galacto-oligosaccharides as a carbon source. Dietary supplement of galacto-oligosaccharides is furthermore thought to have a number of long-term disease protecting effects. For example, galacto-oligosaccharide intake has been shown to be highly protective against development of colorectal cancer in rats (Wijnands, *et al.*, 1999). There is therefore a great interest in developing cheap and efficient methods for producing galacto-oligosaccharides for use in the industry for improving dietary supplements and dairy products.

The enzyme β-galactosidase (EC 3.2.1.23) usually hydrolyses lactose to the monosaccharides D-glucose and D-galactose. In the normal enzyme reaction of β-galactosidases, the enzyme hydrolyses lactose and transiently binds the galactose monosaccharide in a galactose-enzyme complex that transfers galactose to the hydroxyl group of water, resulting in the liberation of D-galactose and D-glucose. However, at high lactose concentrations some β-galactosidasees are able to transfer galactose to the hydroxyl groups of D-galactose or D-glucose in a process called transgalactylation whereby galacto-oligosaccharides are produced.

Enzymes capable of transgalactosylation have been isolated from a wide range of micro-organisms, including bacteria and yeasts. The observation that galacto-oligosaccharides enhance the growth of health-promoting *Bifidobacterium* has stimulated investigations of *Bifidobacterium* and their β-galactosidase enzymes. Two DNA sequences of *B. breve* and *B. longum* β-galactosidase

genes have been deposited in GeneBank (accession numbers E5040 and AJ242596, respectively). Dumortier *et al.* (1994) have reported that *B. bifidum* DSM 20215 contains three β-galactosidases and one of these enzymes has trans-galactosylating properties. However, no identification of the enzyme possessing this activity or any sequence of the enzyme or the corresponding gene from *B. bifidum* DSM 20215 has been published.

Production of galacto-oligosaccharides by the use of β-galactosidases has been reported in several papers. For example, β-galactosidase from *E. coli* has been shown to produce oligosaccharides at high lactose concentrations (0.5 M or approximately 20% lactose; Huber *et al.* 1976). Various thermophilic microorganisms have been shown to produce oligosaccharides at high temperatures and high lactose concentrations, e.g. *Sterigmatomyces elviae* can produce 39% oligosaccharides from 20% lactose at 60°C (Onishi & Tanaka, 1995), and Saccharopolyspora rectivirgula can synthesize 41% oligosaccharides in 1.75 M lactose at 70°C (Nako *et al.*, 1994).

However, the enzymes described above all have the drawbacks of requiring either high temperatures or high lactose concentrations or both in order to exhibit significant transgalactosylase activity. There is thus a need for developing cheaper and more efficient methods of producing galacto-oligosaccharides for use in the industry.

Summary of the invention

The present invention describes a new β-galactosidase from *Bifidobacterium bifidum*. A truncated version of the

enzyme has surprisingly been shown to have a high transgalactosylating activity. When the truncated enzyme, or a host cell expressing the recombinant truncated enzyme is incubated with lactose under appropriate conditions, galacto-oligosaccharides are produced at a high efficientcy. Presence of galacto-oligosaccharides in diary products or other comestible products have the advantage of enhancing the growth of health-promoting *Bifdobacterium* in the product or in the intestinal flora of the consumer after intake of the product or both.

Brief descrition of the drawings

Figure 1:

OLGA5 sequence. DNA and protein sequence of the OLGA5 β-galactosidase from *Bifidumbacterium bifidum*. The signal sequence is shown in bold and the part of OLGA5 gene deleted in OLGA347 is shown in italics. The *Bgl*II site used to create the deletion is highlighted.

Figure 2:

Comparison of β-galactosidase active site regions. Alignment of regions around the catalytic Glu461 residue (highlighted) from *E. coli*. The sequences are identified by their database accession numbers. 6-phospho-β-galactosidase sequences are marked with a (P).

Figure 3:

Neighbour joining analysis of the alignment in Figure 1, where the *Sulfolubus* sequences were used as an outgroup. Results from a bootstrap analysis (n = 100) are shown for the junctions with a value above 80.

Figure 4:

OLGA5 transgalactosylase activity. Total cell lysate of *E. coli* cells harbouring the OLGA5 gene in a plasmid were incubated with 0.4 M lactose at 37°C for 20 hours. A 50 µl total reaction volume contained the indicated amounts of total cell lysate. Reaction samples were analysed on a silica gel TLC plate. The plate was sprayed with Orcinol reagent to visualise the sugars.

Figure 5:

C-terminal deletions of OLGA5 β-galactosidase. A 1752 amino acid open reading frame encodes the OLGA5 β-galactosidase, where the starting 32 amino acids likely represent a signal peptide (white box). Deletion mutants of OLGA5 were constructed using the indicated restriction sites. Lysates prepared from bacterial cultures grown over night were used for measurement of β-galactosidase activity, and the relative results are shown to the right of the respective constructs. Restriction enzyme symbols used: *Bgl*II (B), *Eco*RI (E), *Eco*RV (V), *Hind*III (H), *Kpn*I (K), *Nru*I (N), *Pst*I (P).

Figure 6:

TLC analysis of transgalactosylase activity. Total cell lysates for the two tested deletion mutants, OLGA347 and OLGA345, were used in the indicated amounts to react with 0.4 M lactose in 50 µl total volume. The reactions were incubated at 37°C for 20 hours. Samples were analysed on a silica gel TLC plate. The plate was sprayed with Orcinol reagent to visualise the sugars.

Figure 7:

Oligosaccharides produced by OLGA347. The indicated amounts of OLGA347 total cell lysate were incubated with 15% lactose in a total volume of µl for 21 hours at 37°C.

Radioactive lactose that was labelled with ^{14}C in the glucose C-1 position was used. Samples were separated on a TLC plate and quantitated by use of a phospho-imager. A: Image used for measurement of ^{14}C-signals from lactose, glucose and galacto-oligosaccharides (GOS) spots. B: Measured ^{14}C-signals after subtraction of background (blind lane).

Figure 8:

HPLC measurement of OLGA347 enzyme reaction products. Reactions in 10%, 20% and 40% lactose were performed using the indicated amounts of OLGA347 total cell lysate. A total volume of 200 µl was used and the reactions were incubated at 37°C for 20 hours. Diluted samples were subjected to HPLC analysis and standard curves were used to convert the observed peak areas to concentrations (mg/ml). A: Obtained mg/ml saccharide after OLGA347 reaction with 10% lactose. B: Obtained mg/ml saccharide after OLGA347 reaction with 20% lactose. C: Obtained mg/ml saccharide after OLGA347 reaction with 40% lactose. D: Plot of results from the 10% reaction. The resulting amount of galacto-oligosaccharides is calculated as the amount of lactose not recovered as glucose or galactose ("GOS").

Detailed description of the invention

The first aspect of the invention concerns a new β-galactosidase, OLGA5 (SEQ ID NO:1 and SEQ ID NO:2), from *Bifidobacterium bifidum* that has been isolated and characterised. *E. coli* cells were transformed with a plasmid containing insertions consisting of *PstI* digested chromosomal DNA from *B. bifidum*. Clones with β-galactosidase activity were selected on plates containing

a chromogenic β-galactosidase substrate. One of the positive colonies contained a plasmid with an insert of approximately 20 kb, pOLGA5 (SEQ ID NO:1). Sequencing of the DNA sequence revealed that the deduced amino acid sequence of OLGA5 β-galactosidase (SEQ ID NO:2) is approximately twice as long as the presently known β-galactosidases and it furthermore shows a surprisingly low degree of sequence homology with known β-galactosidases. Expression of recombinant OLGA5 in *E. coli* revealed that the enzyme, in addition to lactose hydrolysing activity, also exhibited transgalactosylating activity. The C-terminal part of the OLGA5 enzyme showed no homology to known β-galactosidases. A variety of OLGA5 C-terminal deletion mutants were subsequently constructed and the resulting enzymes were investigated for their hydrolytic and transgalactosylating activity.

A second aspect of the invention concerns deletion mutants of OLGA5, e.g. OLGA347. Out of several C-terminal deletion mutants, OLGA347 which has a 578 amino acid C-terminal deletion, showed the most pronounced increased level of oligosaccharides produced when incubated with lactose even at relatively low lactose concentrations. The enzyme apparently transferred virtually all galactose molecules onto galactose or glucose. Deletion of the C-terminal end of OLGA5 hence converted the enzyme from a hydrolytic OLGA5 β-galactosidase to a transgalactosylating OLGA347-transgalactosidase. Unlike other transgalactosylating β-galactosidases, including the native OLGA5 enzyme, the truncated β-galactosidase

OLGA347 transfers galactose onto acceptor sugar molecules at high frequency at all lactose concentrations examined.

In one embodiement, an expression vector with an insert encoding OLGA5, OLGA342, OLGA345, OLGA347, OLGA344, or any other OLGA5 variant is used. This expression vector can be transformed into a host cell selected from the group comprising *Bifidobacterium, Lactococcus, Lactobacillus, Streptococcus, Leuconostoc, Escherichia, Bacillus, Streptomyces, Saccharomyces, Kluyveromyces, Candida, Torula, Torulopsis* and *Aspergillus*. A cell of the genus *Bifidobacterium* is selected from the group consisting of *Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium bifidum* and *Lactococcus lactis*. The cell is then cultured in a suitable culture medium under conditions permitting expression of for example an OLGA5 or an OLGA347 variant and the resulting enzyme is thereafter recovered from the culture.

In another embodiment of the invention, an OLGA5 variant is part of an expression vector, which can be transformed into any one of the above, mentioned host cells. The cell is then cultured in a suitable culture medium under conditions permitting expression of the OLGA5 variant and the resulting enzyme is thereafter recovered from the culture. The OLGA5 variant may contain any random mutation or any mutation generated by conventional molecular biology techniques. Any fragment of a mutated or a wild-type OLGA5 DNA molecule can be inserted into the expression vector. The fragment can be generated by PCR (polymerase chain reaction) or by means of any restriction sites present in the sequence or a combination of both. The procedures for generating OLGA5

variants are well known to a person skilled in the art. It is thus not critical to the present invention in which way the variant is obtained. The variants disclosed in the present text are obtained by subcloning by use of restriction sites present in the sequence.

Another aspect of the invention concerns use of one or more of the above mentioned cell types for producing a product selected from the group consisting of yoghurt, cheese, fermented dairy products, dietary supplements and probiotic comestible products. In this aspect, the technical effect of the enhanced growth of *Bifidobacterium* is used for improving the quality of the industrial products. Addition of galacto-oligosaccharides enhances the growth of health-promoting *Bifidobacterium*. Galacto-oligosaccharides produced by OLGA347 is thus much cheaper and easier to obtain compared to using native β-galactosidases for producing oligosaccharides.

Yet another aspect of the invention concerns the use of OLGA5, OLGA342, OLGA345, OLGA347, OLGA344 or any other OLGA5 variant or the use of any one or more of the above mentioned cell types for producing oligosaccharides. The oligosaccharides comprise, but are not limited to fructooligo-saccharides, galacto-oligosaccharides, isomalto-oligosaccharides, malto-oligosaccharides, lacto-sucrose and xylo-oligosaccharides.

In one embodiment of the invention, the oligosaccharides are produced by incubating the cell expressing the OLGA5 variant in a medium that comprises a disaccharide substrate such as for example lactulose, trehalose, rhamnose, maltose, sucrose, lactose, or cellobiose. The

incubation is carried out under conditions where oligosaccarides are produced. The cells may be part of a product selected from the group consisting of yoghurt, cheese, fermented milk products, dietary supplements, and probiotic comestible products. Alternatively, the oligo-saccharides can be recovered and subsequently be added to the product of interest before or after its preparation. Addition of oligosaccharides enhance growth of either *Bifidobacterium* alone or of *Bifidobacterium* in a mixed culture.

In another embodiment, the oligosaccharides are produced by incubating the OLGA5 variant in a medium that comprises a disaccharide substrate such as for example lactulose, trehalose, rhamnose, maltose, sucrose, lactose, or cellobiose. The incubation is carried out under conditions where oligosaccarides are produced. The medium comprising an OLGA5 variant and lactose may be part of a product selected from the group consisting of yoghurt, cheese, fermented milk products, dietary supplements, and probiotic comestible products. Alternatively, the oligo-saccharides can be recovered and subsequently be added to the product of interest before or after its preparation. Addition of oligosaccharides enhances growth of either *Bifidobacterium* alone or of *Bifidobacterium* in a mixed culture.

Definitions

"β-galactosidase or a fragment thereof". β-galactosidase is defined as an enzyme capable of hydrolysing lactose to the monosaccharides D-glucose and D-galactose. A fragment of the β-galactosidase comprises 5-98%, preferably 40-95%

and most preferably 55-75% of the protein and the deletion preferably concerns the C-terminal end.

A "host cell" is selected from the group consisting of: fungi, yeasts, and prokaryotes. The micro-organism is more preferably a prokaryote and most preferably a bacterium of the genus *Bifidobacterium* or the species *E. coli*.

By "oligosaccharides" is meant an oligosaccharide consisting of at least three sugar molecules. An example of an oligosaccharide, which is not meant to be limiting, is galacto-oligosaccharide. The linkages between the sugar residues of the oligosaccharide comprise but are not limited to 1-4 and 1-6 bindings.

Incubation of β-galactosidase with lactose takes place in the presence of 0.5-60% lactose, preferably 2-30% lactose and most preferably 2-15% lactose.

Conditions of incubating β-galactosidase with lactose are defined by performing the incubation at a temperature between 5 and 75 °C, preferably 15-45 °C, and most preferably at 37 °C. The time required for the incubation is 1-50 hours, preferably 5-40 hours and most preferably 15-25 hours.

A "comestible product" comprises a product intended for ingestion such as foods, drinks, tablets, and powders.

Examples

Example 1:

Isolation and characterisation of transgalactosylating β-galactosidase from *B. bifidum*. *Pst*I digested chromosomal DNA from *B. bifidum* DSM 20215 was ligated into pKS plasmid (Stratagene) using standard procedures. The ligation mixture was transformed into *E. coli* strain MT102 defective in LacZ and β-galactosidase. β-galactosidase producing clones were identified as blue colonies on plates containing the chromogenic β-galactosidase substrate X-gal.

One of the blue colonies contained a plasmid with an insert of approximately 20 kb, pOLGA5. The insert was further subcloned and partly sequenced and an open reading frame encoding a putative β-galactosidase (OLGA5 β-galactosidase) was identified (Figure 1). BLAST search showed that OLGA5 β-galactosidase showed the highest degree of homology with *Streptomyces coelicolor* β-galactosidase (AL133171) and *Thermoanaerobacter ethanolicus* (YO8557) with 38% and 30% identity, respectively. Figure 3 shows an "identity tree" of OLGA5 and related amino acid sequences.

A detailed analysis of the amino acid sequence of OLGA5 β-galactosidase revealed that the enzyme contains a putative signal sequence at its N-terminal and that the open reading frame encodes a polypeptide of 185 kDa which is approximately twice as large as any of the presently known β-galactosidases. Recombinant OLGA5 enzyme produced in *E. coli* was purified and N-terminal amino acid sequencing confirmed, that the signal sequence was

cleaved during expression in *E. coli*. SDS-PAGE confirmed the molecular weight of the OLGA5 polypeptide.

Cellular extracts of recombinant *E. coli* MT102 containing pOLGA5 were prepared and analysed for transgalactosylating activity. Figure 4 shows that OLGA5, in addition to lactose hydrolysing activity, also exhibited transgalactosylating activity.

Example 2

Construction of a truncated OLGA5 β-galactosidase with high transgalactosylase activity. The region of OLGA5 homologous to other β-galactosidases is located in the N-terminal end of the protein. The C-terminal half showed no homology to any known β-galactosidase. However, a sialidase-like galactose-binding domain was observed in the C-terminal part. The role of this C-terminal part of the OLGA5 β-galactosidase was investigated by construction of truncated deletion mutants. The hydrolytic and transgalactosylating activities of the resulting recombiant β-galactosidases were analysed. Figure 5 shows that it was possible to delete almost one third of the OLGA5 enzyme and still retain hydrolytic activity.

When the transgalactosylating activity was analysed, similar results were obtained with extracts from *E. coli* containing the plasmids pOLGA5, pOLGA342, and pOLGA345. However, extracts of cells harbouring pOLGA347 showed an increased level of oligosaccharides produced and almost no galactose. As shown in Figure 5, an extract containing the truncated OLGA347 β-galactosidase did hydrolyse lactose, but instead of transferring galactose onto

hydroxyl groups in water, the enzyme transferred virtually all galactose molecules onto galactose or glucose (or glycerol; the spot migrating slightly slower than glucose on TLC was shown by NMR to be galacto-glycerol - data not shown). In conclusion OLGA347 is a true "transgalactosylase".

Example 3

Characterisation of the transgalactosylating activity of OLGA347. Two methods were used to quantitate the transgalactosyalting activity of OLGA347 β-galactosidase: TLC analysis of reaction mixtures containing radioactively labelled lactose and HPLC analysis after enzymatic conversion of unlabeled lactose.

Experiments with radioactivity were carried out with lactose containing the ^{14}C-label at the C-1 position of glucose. Since the label was in the glucose part of the disaccharide, only reaction products containing glucose were detected. Figure 7 shows the result of a transgalactosylation experiment with 15% lactose and varying amounts of OLGA347 enzyme. After separation of the reaction mixture by TLC, the plate was scanned and the radioactive spots were quantitated in a phospho-imager. At low enzyme concentrations (between 0 and 0.2 µl of the extract), the glucose and oligosaccharide levels were almost identical, indicating that all glucose molecules were exploited as substrate in transgalactosylation reactions. "Free" hydrolysed glucose appeared only at high enzyme concentratins.

In experiments with unlabelled lactose different substrate and enzyme concentrations were examined. Figure 8 shows an experiment in which 10%, 20%, and 40% lactose was used as substrate in enzyme reactions with varying concentrations of OLGA347 enzyme. The reaction mixtures were analysed with HPLC and the concentrations of lactose, glucose, galactose, and galacto-oligosaccharides were calculated. Figure 8 shows that as the enzyme concentrations goes up, the lactose concentration is decreased and the glucose concentration is increased but virtually no "free" galactose is produced, indicating that almost all galactose molecules in lactose are transferred onto another sugar. Calculations of carbohydrate concentrations measured in reactions with low enzyme concentrations, indicated that the ratio between glucose and galactose is approximately 0.1, implying that for every lactose molecule hydrolysed to free galactose and glucose, nine lactose molecules are used in transglactosylation. As seen in Figure 8, the transgalactosylation reaction is independent of lactose concentration in range from 10% to 40% lactose. The maximal yield of galacto-oligosaccharides produced in transgalactosylation reactions with 10%, 20% or 40% lactose as substrate were 39%, 44% and 37% respectively (mg of oligosaccharides produced per mg lactose added).

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Claims

1. A DNA sequence which

   a) encodes a protein with an amino acid sequence as given in SEQ ID NO:2, or
   b) hybridises under stringent conditions to the sequence of a), or
   c) is degenerative of the sequence of a) or b).

2. A DNA sequence according to claim 1, wherein the sequence is as given in SEQ ID NO:1 or a fragment thereof.

3. A DNA sequence according to claim 2, wherein the sequence comprises a sequence from SEQ ID NO:1 which starts with ATG in position 212-214 and ends with TGA in position 5468-5470, or any fragment thereof.

4. A DNA sequence according to claim 3, wherein the sequence comprises a sequence from SEQ ID NO:1 which starts with ATG in position 212-214 and ends with ATCT in position 3731-3734, or any fragment thereof.

5. A DNA sequence according to claim 3, wherein the the sequence comprises a sequence from SEQ ID NO:1 which starts with GTC in position 308-310 and ends with TGA in position 5468-5470, or any fragment thereof.

6. A DNA sequence according to claim 3, wherein the sequence comprises a sequence from SEQ ID NO:1 which starts with GTC in position 308-310 and ends with ATCT in position 3731-3734, or any fragment thereof.

7. A DNA sequence according to any one of claims 1-6, wherein said sequence comprises nucleotide substitutions, additions or deletions which result in less than 60%, preferably less than 45%, more preferably less than 25% change in the amino acid sequence according to SEQ ID NO:2, or a fragment thereof.

8. A DNA sequence according to any one of claims 1-5, wherein said sequence comprises nucleotide substitutions, which results in conservative amino acid substitutions.

9. An enzyme encoded by a DNA sequence of any one of claims 1-8.

10. An enzyme comprising an amino acid sequence according to SEQ ID NO:2, or a fragment thereof.

11. A β-galactosidase having the sequence as defined in SEQ ID NO:2.

12. An enzyme according to claim 10 having the sequence as defined in SEQ ID NO:2 from Met (1) to Gly (1752), or a fragment thereof.

13. A mature β-galactosidase according to claim 12.

14. An enzyme according to claim 10 having the sequence as defined in SEQ ID NO:2 from Met (1) to Ile (1174), or a fragment thereof.

15. A transgalactosylating enzyme according to claim 14.

16. An enzyme according to claim 14 having the sequence as defined in SEQ ID NO:2 from Ala (33) to Ile (1174), or a fragment thereof.

17. A mature transgalactosylating enzyme according to claim 16.

18. A transgalactosylating enzyme of any one of claims 14-17 having one or more of the following characteristics:

a) The ratio of transgalactosylating activity to β-galactosidase activity in a solution of 100 g/L lactose at 37 °C is at least 1:1,
b) catalyses production of at least 25% galacto-oligosaccharides in batch reaction with a solution of 100 g/L lactose at 37 °C,
c) catalyses production of galacto-oligo-saccharides in batch reaction with a solution of 100 g/L lactose at 37 °C with less than 15% of galactose from the lactose being present in the free form at the reaction time with maximum concentration of galacto-oligo-saccharide.

19. A recombinant vector comprising a DNA sequence of any one of claims 1-8.

20. A vector of claim 19, wherein said vector is an expression vector.

21. A host cell comprising a DNA sequence of any one of claims 1-8.

22. A host cell comprising a vector of any one of claims 19-20.

23. A cell of claims 21-22, wherein said cell is a bacterial cell, a yeast cell, or a fungal cell.

24. A cell of claim 23, wherein the cell is selected from the group consisting of *Bifidobacterium*, *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Escherichia*, *Bacillus*, *Streptomyces*, *Saccharomyces*, *Kluyveromyces*, *Candida*, *Torula*, *Torulopsis* and *Aspergillus*.

25. A cell of claim 24, wherein the cell is selected from the group consisting of *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium bifidum* and *Lactococcus lactis*.

26. Use of a cell of any one of claims 21-25 for producing a product selected from the group consisting of yoghurt, cheese, fermented milk product, dietary supplement and probiotic comestible product.

27. A dairy product comprising a cell of any one of claims 21-25.

28. Use of a transgalactosylating enzyme of any one of claims 14-18 or a cell of any one of claims 21-25, for producing galacto-oligosaccharides.

29. Use of a transgalactosylating enzyme of any one of claims 14-18 or a cell of any one of claims 21-25, for producing galacto-oligosaccharides to be part of a product selected from the group consisting of yoghurt, cheese, fermented dairy products, dietary supplements and probiotic comestible products.

30. Use of a transgalactosylating enzyme of any one of claims 14-18 or a cell of any one of claims 21-25, for producing galacto-oligosaccharides to enhance the growth of *Bifidobacterium*.

31. Use of a transgalactosylating enzyme of any one of claims 14-18 or a cell of any one of claims 21-25, for producing galacto-oligosaccharides to enhance the growth of *Bifidobacterium* in a mixed culture fermentation.

32. A process for producing a transgalactosylating enzyme of any one of claims 14-18, comprising culturing a cell of any one of claims 21-25 in a suitable culture medium under conditions permitting expression of said enzyme, and recovering the resulting enzyme from the culture.

33. A process for producing galacto-oligosaccharides, comprising contacting of an enzyme of any one of claims 14-18 or a cell of any one of claims 21-25 with a solution of lactose.

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   1  ATGCGTTGCGTTGCGATTTTTCCGGCCCTGTATGGGGGATACAGGATTGGCGATGGCGACACGCCGTTTTTGTTAATGGC
  81  ATTTACATGAAATACAGGTAATGAGATATCATTCTCATGATCACCGTGTGGATATCGCATTGGTGCGTATACACTAACAG

 161  CAACAGAGCGGCGCGGCAGGCGCTCGTGGATTCAATGAAGAAGGAACGTTTATGGCAGTTCGCAGACTTGGTGGCCGCAT
                                                          M  A  V  R  R  L  G  G  R  I

 241  CGTGGCTTTCGCCGCCACAGTGGCCTTGTCAATACCGTTAGGGTTGTTAACAAATTCAGCGTGGGCGGTCGAGGACGCCA
        V  A  F  A  A  T  V  A  L  S  I  P  L  G  L  L  T  N  S  A  W  A  V  E  D  A

 321  CCCGATCCGACTCCACCACGCAGATGAGCTCCACGCCGGAGGTGGTCTACTCCAGCGCCGTGGATTCCAAGCAGAATCGC
        T  R  S  D  S  T  T  Q  M  S  S  T  P  E  V  V  Y  S  S  A  V  D  S  K  Q  N  R

 401  ACCTCGGATTTCGACGCCAACTGGAAGTTCATGCTGTCCGATTCCGTGCAGGCGCAGGATCCGGCGTTCGACGATTCGGC
        T  S  D  F  D  A  N  W  K  F  M  L  S  D  S  V  Q  A  Q  D  P  A  F  D  D  S  A

 481  CTGGCAGCAGGTCGACCTGCCGCATGACTACAGCATCACGCAGAAGTATTCGCAGAGCAACGAGGCCGAAAGCGCATACC
          W  Q  Q  V  D  L  P  H  D  Y  S  I  T  Q  K  Y  S  Q  S  N  E  A  E  S  A  Y

 561  TTCCCGGCGGCACCGGCTGGTACCGCAAGTCCTTCACCATCGACCGGGACCTCGCCGGCAAGCGCATCGCCATCAACTTC
        L  P  G  G  T  G  W  Y  R  K  S  F  T  I  D  R  D  L  A  G  K  R  I  A  I  N  F

 641  GACGGCGTGTACATGAACGCCACCGTCTGGTTCAACGGCGTCAAGCTCGGCACCCATCCGTACGGCTACTCGCCGTTCTC
        D  G  V  Y  M  N  A  T  V  W  F  N  G  V  K  L  G  T  H  P  Y  G  Y  S  P  F  S

 721  CTTCGACCTGACCGGCAACGCCAAGTTCGGTGGGGAGAACACCATCGTCGTCAAGGTCGAGAACAGGCTGCCGTCCAGCC
          F  D  L  T  G  N  A  K  F  G  G  E  N  T  I  V  V  K  V  E  N  R  L  P  S  S

 801  GCTGGTACTCCGGCTCCGGCATCTACCGCGACGTCACCCTCACCGTCACCGACGGCGTGCACGTCGGCAATAACGGCGTG
        R  W  Y  S  G  S  G  I  Y  R  D  V  T  L  T  V  T  D  G  V  H  V  G  N  N  G  V

 881  GCCATCAAGACCCCGAGCCTCGCCACCCAAAACGGCGGCGACGTGACGATGAACCTCACCACCAAGGTCGCCAACGACAC
        A  I  K  T  P  S  L  A  T  Q  N  G  G  D  V  T  M  N  L  T  T  K  V  A  N  D  T

 961  CGAGGCCGCGGCGAACATCACCCTCAAGCAGACCGTGTTCCCCAAGGGAGGCAAGACCGACGCCGCCATCGGCACCGTCA
          E  A  A  A  A  N  I  T  L  K  Q  T  V  F  P  K  G  G  K  T  D  A  A  I  G  T  V

1041  CCACCGCATCCAAGTCCATCGCGGCCGGTGCCAGCGCGGACGTGACCTCCACGATCACCGCCGCTTCGCCCAAGCTGTGG
        T  T  A  S  K  S  I  A  A  G  A  S  A  D  V  T  S  T  I  T  A  A  S  P  K  L  W

1121  AGCATCAAGAACCCGAACCTGTACACCGTGCGCACCGAAGTGCTCAACGGCGGCAAGGTGCTCGACACTTACGACACCGA
        S  I  K  N  P  N  L  Y  T  V  R  T  E  V  L  N  G  G  K  V  L  D  T  Y  D  T  E

1201  ATATGGCTTCCGCTGGACCGGCTTCGATGCGACCAGCGGTTTCTCGCTCAACGGCGAGAAAGTCAAGCTCAAGGGCGTCT
          Y  G  F  R  W  T  G  F  D  A  T  S  G  F  S  L  N  G  E  K  V  K  L  K  G  V

1281  CAATGCATCATGACCAGGGATCGCTCGGCGCGGTCGCCAACCGCCGCGCCATCGAGCGCCAGGTCGAGATTCTCCAGAAG
        S  M  H  H  D  Q  G  S  L  G  A  V  A  N  R  R  A  I  E  R  Q  V  E  I  L  Q  K

1361  ATGGGCGTCAACTCGATCCGCACCACGCACAACCCCGCAGCCAAGGCGCTGATTGACGTCTGCAACGAGAAGGGCGTCCT
          M  G  V  N  S  I  R  T  T  H  N  P  A  A  K  A  L  I  D  V  C  N  E  K  G  V  L

1441  CGTGGTCGAAGAGGTCTTCGACATGTGGAACCGGTCGAAGAACGGCAACACCGAGGATTACGGCAAGTGGTTCGGCCAGG
        V  V  E  E  V  F  D  M  W  N  R  S  K  N  G  N  T  E  D  Y  G  K  W  F  G  Q

1521  CCATCGCCGGTGACAACGCCGTCCTGGGTGGCGACAAGGACGAGACCTGGGCCAAGTTCGACCTGACCAGCACCATCAAC
        A  I  A  G  D  N  A  V  L  G  G  D  K  D  E  T  W  A  K  F  D  L  T  S  T  I  N

1601  CGTGACAGGAACGCCCCGTCCGTCATCATGTGGTCGCTCGGCAACGAGATGATGGAAGGCATCAGCGGCAGCGTCTCGGG
          R  D  R  N  A  P  S  V  I  M  W  S  L  G  N  E  M  M  E  G  I  S  G  S  V  S  G

1681  CTTCCCGGCTACCTCCGCCAAGCTGGTCGCATGGACGAAGGCCGCGGACAGCACCCGCCCGATGACCTACGGCGACAACA
          F  P  A  T  S  A  K  L  V  A  W  T  K  A  A  D  S  T  R  P  M  T  Y  G  D  N

1761  AGATCAAGGCCAACTGGAACGAGTCGAACACCATGGGCGACAACCTGACCGCCAACGGCGGCGTGGTCGGCACCAACTAC
        K  I  K  A  N  W  N  E  S  N  T  M  G  D  N  L  T  A  N  G  G  V  V  G  T  N  Y

1841  TCCGACGGCGCGAACTACGACAAGATCCGCACGACCCACCCCTCATGGGCCATCTATGGTTCCGAGACGGCGTCCGCCAT
          S  D  G  A  N  Y  D  K  I  R  T  T  H  P  S  W  A  I  Y  G  S  E  T  A  S  A  I

1921  CAACAGCCGAGGCATCTACAACCGCACCACCGGCGGCGCCCAGTCAAGCGACAAGCAGCTGACCAGCTATGACAATTCCG
          N  S  R  G  I  Y  N  R  T  T  G  G  A  Q  S  S  D  K  Q  L  T  S  Y  D  N  S

2001  CAGTCGGCTGGGGCGCCGTCGCCAGCTCCGCCTGGTACGACGTGGTCCAGCGCGATTTCGTCGCCGGCACATACGTGTGG
        A  V  G  W  G  A  V  A  S  S  A  W  Y  D  V  V  Q  R  D  F  V  A  G  T  Y  V  W

2081  ACCGGCTTCGACTACCTCGGCGAACCCACCCCGTGGAACGGCACCGGCTCCGGCGCCGTGGGCTCCTTGGCCGTCGCCGA
          T  G  F  D  Y  L  G  E  P  T  P  W  N  G  T  G  S  G  A  V  G  S  L  A  V  A  E

2161  AGAACTCGTACTTCGGCATCGTCGACACCGCAGGCTTCCCGAAGACACCTATTACTTCTATCAGAGCCAGTGGAACGACG
          E  L  V  L  R  H  R  R  H  R  R  L  P  E  D  T  Y  Y  F  Y  Q  S  Q  W  N  D

2241  ACGTGCACACGCTGCACATCCTCCCCGCATGGAACGAGAACGTCGTCGCCAAGGGCTCCGGCAACAACGTGCCGGTCGTC
        D  V  H  T  L  H  I  L  P  A  W  N  E  N  V  V  A  K  G  S  G  N  N  V  P  V  V
```

Fig. 1

```
2321  GTCTACACCGACGCGGCCAAGGTCAAGCTGTACTTCACACCGAAGGGCAGTACCGAAAAGCGACTGATCGGAGAGAAGTC
        V  Y  T  D  A  A  K  V  K  L  Y  F  T  P  K  G  S  T  E  K  R  L  I  G  E  K  S

2401  CTTCACCAAGAAGACCACCGCGGCCGGATACACCTATCAGGTCTACGAGGGCTCCGACAAGGACTCCACCGCCCACAAGA
        F  T  K  K  T  T  A  A  G  Y  T  Y  Q  V  Y  E  G  S  D  K  D  S  T  A  H  K

2481  ACATGTACCTGACCTGGAACGTGCCGTGGGCCGAGGGCACCATCTCCGCCGAAGCATACGACGAGAACAACAGGCTGATC
      N  M  Y  L  T  W  N  V  P  W  A  E  G  T  I  S  A  E  A  Y  D  E  N  N  R  L  I

2561  CCCGAGGGGTCCACCGAGGGCAACGCGTCGGTGACCACCACCGGCAAGGCCGCGAAGCTTAAAGCCGATGCCGACCGCAA
        P  E  G  S  T  E  G  N  A  S  V  T  T  T  G  K  A  A  K  L  K  A  D  A  D  R  K

2641  GACGATCACCGCGGACGGCAAGGACCTGTCGTACATCGAGGTCGACGTGACCGACGCCAACGGCCATATCGTCCCCGATG
        T  I  T  A  D  G  K  D  L  S  Y  I  E  V  D  V  T  D  A  N  G  H  I  V  P  D

2721  CCGCCAACCGCGTCACCTTCGACGTCAAGGGCGCCGGCAAACTGGTCGGCGTCGACAACGGCAGCTCGCCGGATCACGAC
      A  A  N  R  V  T  F  D  V  K  G  A  G  K  L  V  G  V  D  N  G  S  S  P  D  H  D

2801  TCCTATCAGGCCGACAACCGCAAGGCGTTCAGCGGCAAGGTGCTCGCCATCGTCCAGTCCACCAAGGAGGCGGGCGAGAT
        S  Y  Q  A  D  N  R  K  A  F  S  G  K  V  L  A  I  V  Q  S  T  K  E  A  G  E  I

2881  CACCGTCACCGCCAAGGCCGACGGTCTGCAATCATCCACAGTGAAGATCGCCACCACCGCCGTCCCCGGCACCAGCACCG
        T  V  T  A  K  A  D  G  L  Q  S  S  T  V  K  I  A  T  T  A  V  P  G  T  S  T

2961  AGAAGACGGTCCGCAGCTTCTACTACTCGCGCAACTACTACGTCAAGACCGGCAACAAGCCGATTCTGCCGAGTGATGTC
      E  K  T  V  R  S  F  Y  Y  S  R  N  Y  Y  V  K  T  G  N  K  P  I  L  P  S  D  V

3041  GAGGTGCGCTACTCCGACGGCACGTCGGACCGTCAGAACGTCACATGGGACGCAGTCAGCGACGACCAGATCGCCAAGGC
        E  V  R  Y  S  D  G  T  S  D  R  Q  N  V  T  W  D  A  V  S  D  D  Q  I  A  K  A

3121  CGGTTCGTTCAGCGTGGCCGGCACGGTCGCCGGGCAGAAGATCTCCGTGCGCGTGACGATGATCGACGAGATCGGTGCGC
        G  S  F  S  V  A  G  T  V  A  G  Q  K  I  S  V  R  V  T  M  I  D  E  I  G  A

3201  TGCTCAACTATTCGGCCAGCACACCGGTCGGCACGCCCGCCGTGCTGCCTGGCTCGCGTCCGGCCGTGCTGCCCGACGGC
      L  L  N  Y  S  A  S  T  P  V  G  T  P  A  V  L  P  G  S  R  P  A  V  L  P  D  G

3281  ACCGTGACCAGCGCGAACTTCGCCGTCCACTGGACCAAGCCCGCCGACACCGTGTACAACACGGCCGGCACCGTCAAGGT
        T  V  T  S  A  N  F  A  V  H  W  T  K  P  A  D  T  V  Y  N  T  A  G  T  V  K  V

3361  CCCCGGCACCGCCACCGTCTTCGGCAAGGAGTTCAAGGTCACCGCGACGATTCGCGTGCAGCGGTCGCAGGTCACCATCG
        P  G  T  A  T  V  F  G  K  E  F  K  V  T  A  T  I  R  V  Q  R  S  Q  V  T  I

3441  GCAGCAGCGTCTCCGGCAATGCGCTGCGCCTGACTCAGAACATCCCCGCCGACAAGCAGTCCGACACGCTGGACGCCATC
      G  S  S  V  S  G  N  A  L  R  L  T  Q  N  I  P  A  D  K  Q  S  D  T  L  D  A  I

3521  AAGGACGGCTCCACGACCGTCGACGCCAATACCGGCGGCGGCGCGAACCCGTCAGCATGGACCAACTGGGCGTACTCGAA
        K  D  G  S  T  T  V  D  A  N  T  G  G  G  A  N  P  S  A  W  T  N  W  A  Y  S  K

3601  GGCCGGCCACAACACCGCCGAGATCACCTTCGAGTACGCGACCGAGCAGCAGCTCGGCCAGATTGTCATGTACTTCTTCC
        A  G  H  N  T  A  E  I  T  F  E  Y  A  T  E  Q  Q  L  G  Q  I  V  M  Y  F  F

3681  GCGACAGCAACGCGGTGAGGTTCCCCGACGCCGGCAAGACGAAGATCCAGATCTCCGCGGACGGCAAGAACTGGACGGAT
      R  D  S  N  A  V  R  F  P  D  A  G  K  T  K  I  Q  I  S  A  D  G  K  N  W  T  D

3761  CTCGCTGCCACGGAGACCATCGCGGCCCAGGAGTCGTCCGACCGAGTCAAGCCGTACACCTATGACTTCGCTCCGGTGGG
        L  A  A  T  E  T  I  A  A  Q  E  S  S  D  R  V  K  P  Y  T  Y  D  F  A  P  V  G

3841  AGCCACGTTCGTCAAGGTCACGGTCACCAACGCCGACACCACAACCCCCAGCGGCGTGGTCTGCGCCGGCCTGACCGAGA
        A  T  F  V  K  V  T  V  T  N  A  D  T  T  T  P  S  G  V  V  C  A  G  L  T  E

3921  TCGAGCTGAAGACCGCGACCAGCAAGTTCGTCACGAACACGTCCGCCGCGCTCTCGTCGCTGACAGTGAACGGCACGAAG
      I  E  L  K  T  A  T  S  K  F  V  T  N  T  S  A  A  L  S  S  L  T  V  N  G  T  K

4001  GTCTCCGACTCCGTGCTCGCCGCCGGCTCCTACAACACGCCCGCGATCATCGCGGACGTCAAAGCCGAGGGCGAAGGCAA
        V  S  D  S  V  L  A  A  G  S  Y  N  T  P  A  I  I  A  D  V  K  A  E  G  E  G  N

4081  CGCCAGCGTCACCGTGCTGCCCGCGCACGACAACGTGATCCGCGTGATCACCGAGTCCGAGGACCACGTCACGCGCAAGA
        A  S  V  T  V  L  P  A  H  D  N  V  I  R  V  I  T  E  S  E  D  H  V  T  R  K

4161  CCTTCACCATCAACCTGGGCACGGAGCAGGAATTCCCCGCAGACTCCGATGAACGCGACTACCCGGCCGCCGACATGACG
      T  F  T  I  N  L  G  T  E  Q  E  F  P  A  D  S  D  E  R  D  Y  P  A  A  D  M  T

4241  GTCACCGTGGGCAGCGAACAGACGTCCGGCACCGCGACCGAAGGCCCGAAGAAATTCGCGGTCGACGGCAACACCAGCAC
        V  T  V  G  S  E  Q  T  S  G  T  A  T  E  G  P  K  K  F  A  V  D  G  N  T  S  T

4321  GTACTGGCATTCCAACTGGACGCCCACCACCGTGAACGACCTGTGGATCGCCTTCGAGCTCCAGAAACCCACCAAGCTCG
        Y  W  H  S  N  W  T  P  T  T  V  N  D  L  W  I  A  F  E  L  Q  K  P  T  K  L

4401  ACGCGCTGCGCTACCTGCCGCGCCCCGCGGGCAGCAAGAACGGCTCCGTCACCGAATACAAGGTTCAGGTCAGCGATGAC
      D  A  L  R  Y  L  P  R  P  A  G  S  K  N  G  S  V  T  E  Y  K  V  Q  V  S  D  D

4481  GGCACCAACTGGACCGACGCGGGCTCCGGCACATGGACCACCGATTACGGCTGGAAGCTCGCCGAGTTCAATCAGCCGGT
        G  T  N  W  T  D  A  G  S  G  T  W  T  T  D  Y  G  W  K  L  A  E  F  N  Q  P  V
```

Fig. 1 (continued)

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4561  GACCACCAAGCACGTGCGGCTCAAGGCCGTCCACACCTATGCGGATTCCGGCAACGACAAGTTCATGTCCGCCTCCGAAA
        T  T  K  H  V  R  L  K  A  V  H  T  Y  A  D  S  G  N  D  K  F  M  S  A  S  E

4641  TCCGCCTGCGCAAGGCCGTCGACACCACCGACATCAGCGGCGCGACCGTGACCGTGCCCGCCAAGCTGACCGTCGACCGG
      I  R  L  R  K  A  V  D  T  T  D  I  S  G  A  T  V  T  V  P  A  K  L  T  V  D  R

4721  GTGGACGCCGACCATCCCGCCACCTTCGCCACGAAGGACGTGACGGTGACGTTGGGCGACGCCACGCTGCGCTACGGCGT
      V  D  A  D  H  P  A  T  F  A  T  K  D  V  T  V  T  L  G  D  A  T  L  R  Y  G  V

4801  GGACTACCTGCTCGACTACGCGGGCAACACCGCCGTCGGCAAGGCCACGGTGACCGTGCGCGGCATCGACAAGTACTCCG
        D  Y  L  L  D  Y  A  G  N  T  A  V  G  K  A  T  V  T  V  R  G  I  D  K  Y  S

4881  GCACCGTCGCCAAGACGTTCACCATCGAACTGAAGAACGCCCCGGCGCCGGAACCGACGCTGACCTCGGTGAGCGTCAAG
      G  T  V  A  K  T  F  T  I  E  L  K  N  A  P  A  P  E  P  T  L  T  S  V  S  V  K

4961  ACCAAGCCTTCCAAGCTGACCTATGTGGTCGGCGACGCGTTCGACCCGGCAGGACTGGTGCTGCAGCACGACAGACAGGC
      T  K  P  S  K  L  T  Y  V  V  G  D  A  F  D  P  A  G  L  V  L  Q  H  D  R  Q  A

5041  CGATCGCCCCCCACAGCCACTTGTTGGAGAACAGGCCGACGAACGCGGACTGACGTGCGGAACGCGATGCGATCGCGTTG
        D  R  P  P  Q  P  L  V  G  E  Q  A  D  E  R  G  L  T  C  G  T  R  C  D  R  V

5121  AACAGCTGCGCAAACACGAGAATCGTGAAGCCCATCGTACGGGCCTCGATCATCTGGAATTCGTGGGTGCCGCCGATGGA
      E  Q  L  R  K  H  E  N  R  E  A  H  R  T  G  L  D  H  L  E  F  V  G  A  A  D  G

5201  GCGGTCGGTGAACAGGCCACCTTCAAGGTGCATGTCCATGCCGATCAAGGTGACGGCCGCCATGATGATGCCGATGAACG
      A  V  G  E  Q  A  T  F  K  V  H  V  H  A  D  Q  G  D  G  R  H  D  D  A  D  E  R

5281  CGATATCGATCCACATGTCCCTGTCGATCACGCGGTCGGTGAGCTTGCGCGGGCTGCGTGCCATCACGTCATCGGTCTGC
        D  I  D  P  H  V  P  V  D  H  A  V  G  E  L  A  R  A  A  C  H  H  V  I  G  L

5361  GGGTCGACACCCATCGCCTCAAGGCATCCGGCTTCCAGATCCCCGCCGACGACATGGCCGAGATCGACCGCATCACCGGC
      R  V  D  T  H  R  L  K  A  S  G  F  Q  I  P  A  D  D  M  A  E  I  D  R  I  T  G

5441  TTCCACCGCTTCGAGCGCCACGTCGGCTGACGTGATTGGGCTTCCCCGCTGTCTGGTGCCGGCTCGCGA
        F  H  R  F  E  R  H  V  G  Z
```

Fig. 1 (continued)

```
L35444      RFLAASQAY--LDALAKQVQPLLN-HNGGP-II-AVQVE-NEYGSYAD
M13466      HYCPNHPQL--ITHIKRLVRAIAERYKNHPALK-MWHVN-NEYACHVS
U17417      TISSSAWYYSVGQYAAKMTRALAERYKDHPALA-LWHVD-NELGCHVS
E05040      HWRATSPVF--LDYALNLCRKMAEHYKDNPYVV-SWHVS-NEYGCHNR
OLGA88      HWRPTSPVF--REYALRLCRAMAEHYRDNPYVV-AWHVS-NEYGCHNR
L03424      NSCPNSPTY--RKYSEKIADKLAERYKDHPAVL-VWHIS-NEYGGDCY
L03425      NHCYTSPVY--REKVTAINTKLAERYSDHPAVI-GWHIS-NEFGGDCH
D49537      NHCYTSPIY--REKIAIIDRLLAERYKDHPALI-LWHIS-NEFEGQCY

L20757      RWGGME-TG--GNPERPPHRSSATG--TTRLSY-IWGVRINESQDSHD
M57579      QYIGNS-EW--KKVAEQNLREMITRDWNHPSII-LWGVRINESQDDDA
Y08557      QHIGDE-NW--KNIAKENLKEMILRDRNHPCIF-MWGVRINERLDDHD
OLGA5       AVLGGDKDE--TWAKFD-LTSTINRDRNAPSVI-MWSLG-NEMMEGIS
M63636      NIPASEPEW--LPACLDRANNMFQRDKNHASVI-IWSCG-NESYAGKD
M35107      NVPGSLPQW--QAAVLDRASSMVERDKNHPSVL-IWSCG-NESYAGED
M92281      NVPGDNPHW--PAAVIDRARSNYEWFKNHPSII-FWSLG-NESYAGED
X82287      NVPGSYDEW--EAATLDRARTNFETFKNHVSIL-FWSLG-NESYAGSV
M23530      NVPGDDQHW--LGASLSRVKNMMARDKNHASIL-IWSLG-NESYAGTV
AJ242596    IVPGSKREW--EGACVDRVNSMMRRDYNHPSVL-IWSLG-NESYVGDV
OLGA2       SVPGDDEAW--LGACIDRLDSMILRDRNHPSVL-VWSLG-NESYAGEV
U62625      CYFARDPLF--KKAILDRQQANVERDKNRTSII-IWSLG-NEAGYGAN
Y14599      NIIADDSKF--ETAIIERIEASIMPLKNYSSIV-SWSLG-NESGFGKN
U08186      VTLANRWEW--EKAHFDRIKRMVERDKNHPSII-FWSLG-NEAGDGVN
OLGA1       RPIADNPAW--IAPTVDRAQRSVERDKNHASII-FWSMG-NECAYGCT
M11441      NRLSDDPAW--LPAFSARVTRMVQSNRNHPCII-IWSLG-NESGGGGN
U60828      NRLTNDPTY--LPLMSERVTRMVMRDRNHPSII-IWSLG-NESGYGSN
J01636      NRLTDDPRW--LPAMSERVTRMVQRDRNHPSVI-IWSLG-NESGHGAN
D42077      SRLADDPRW--LPAMSERVTRMVQRDRNHPSII-IWSLG-NESGHGAN

D37882(P)   EGLHEDGDFLTHEKMDDFVEYADYCFKEFPEVK-YWITI-NEIRSVAV
J03479(P)   EVLHKDGDFLNRKTIDYFVDYAEYCFKEFPEVK-YWTTF-NEIGPIGD
L18993(P)   EALHSNGDFLNRENIEHFVNYAEFCFKEFSEVN-YWTTF-NEIGPIGD
M28357(P)   EALHSNGDFLNRENIEHFIDYAAFCFEEFPEVN-YWTTF-NEIGPIGD

M34696      GDFTGPSGWLSTRTVYEFARFSAYIAWKFDDLVDEYSTM-NEPNVVGG
X15950      GDFTGPTGWLNSRTVYEFARFSAYVAWKLDDLASEYATM-NEPNVVWG
```

Fig. 2

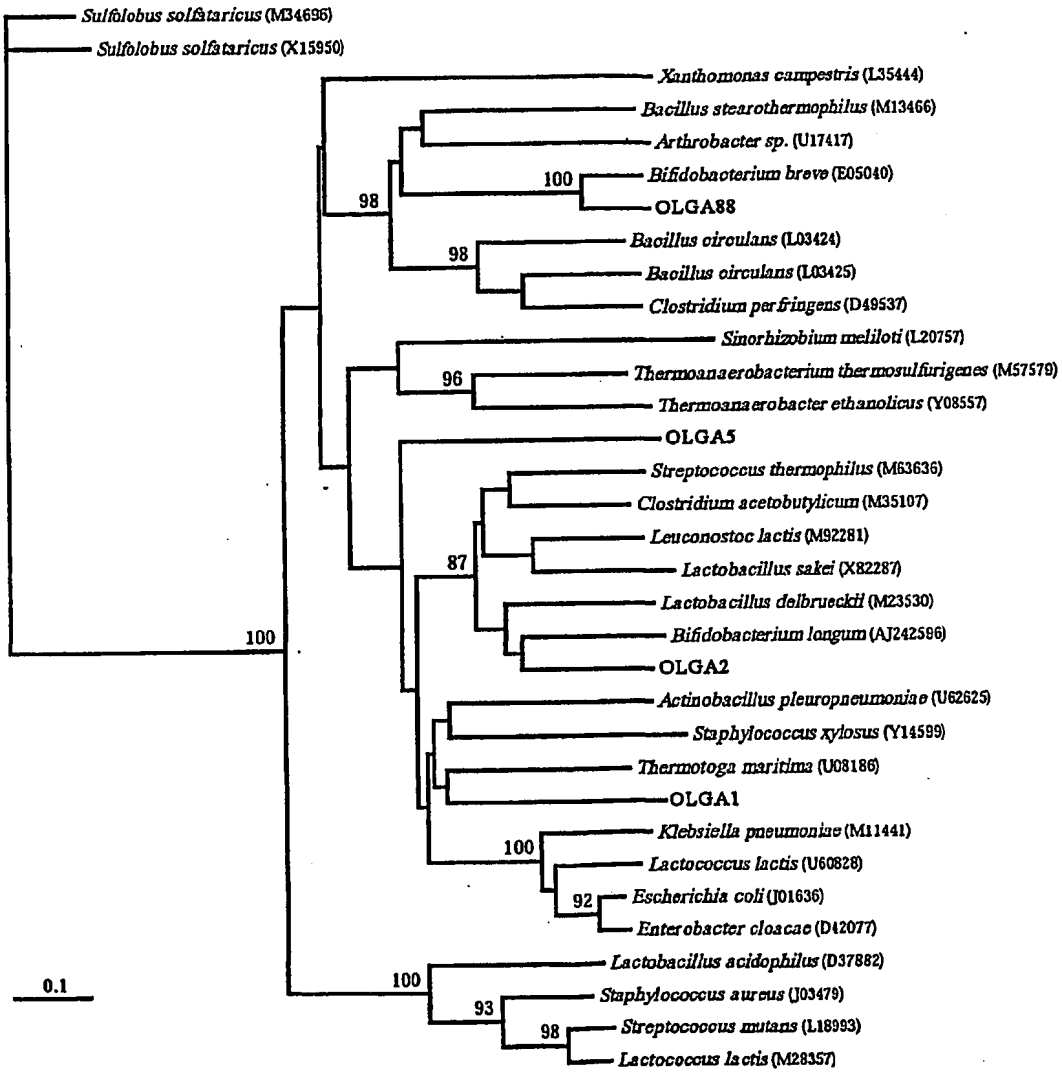

Sulfolobus solfataricus (M34696)
Sulfolobus solfataricus (X15950)
Xanthomonas campestris (L35444)
Bacillus stearothermophilus (M13466)
Arthrobacter sp. (U17417)
Bifidobacterium breve (E05040)
OLGA88
Bacillus circulans (L03424)
Bacillus circulans (L03425)
Clostridium perfringens (D49537)
Sinorhizobium meliloti (L20757)
Thermoanaerobacterium thermosulfurigenes (M57579)
Thermoanaerobacter ethanolicus (Y08557)
OLGA5
Streptococcus thermophilus (M63636)
Clostridium acetobutylicum (M35107)
Leuconostoc lactis (M92281)
Lactobacillus sakei (X82287)
Lactobacillus delbrueckii (M23530)
Bifidobacterium longum (AJ242586)
OLGA2
Actinobacillus pleuropneumoniae (U62625)
Staphylococcus xylosus (Y14599)
Thermotoga maritima (U08186)
OLGA1
Klebsiella pneumoniae (M11441)
Lactococcus lactis (U60828)
Escherichia coli (J01636)
Enterobacter cloacae (D42077)
Lactobacillus acidophilus (D37882)
Staphylococcus aureus (J03479)
Streptococcus mutans (L18993)
Lactococcus lactis (M28357)
100
98
100
98
96
87
100
100
92
100
93
98
0.1

Fig. 3

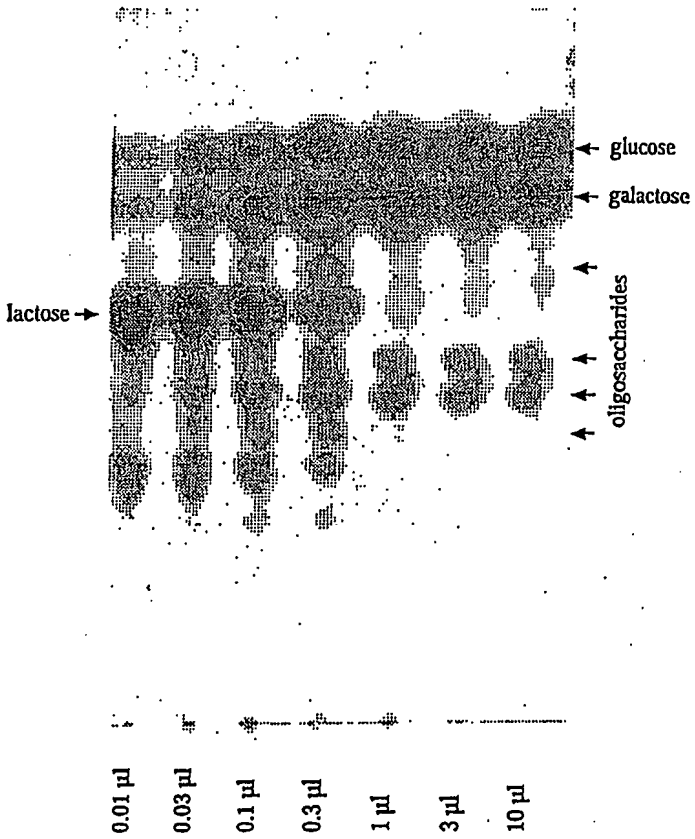

glucose
galactose
lactose
oligosaccharides
0.01 µl
0.03 µl
0.1 µl
0.3 µl
1 µl
3 µl
10 µl

Fig. 4

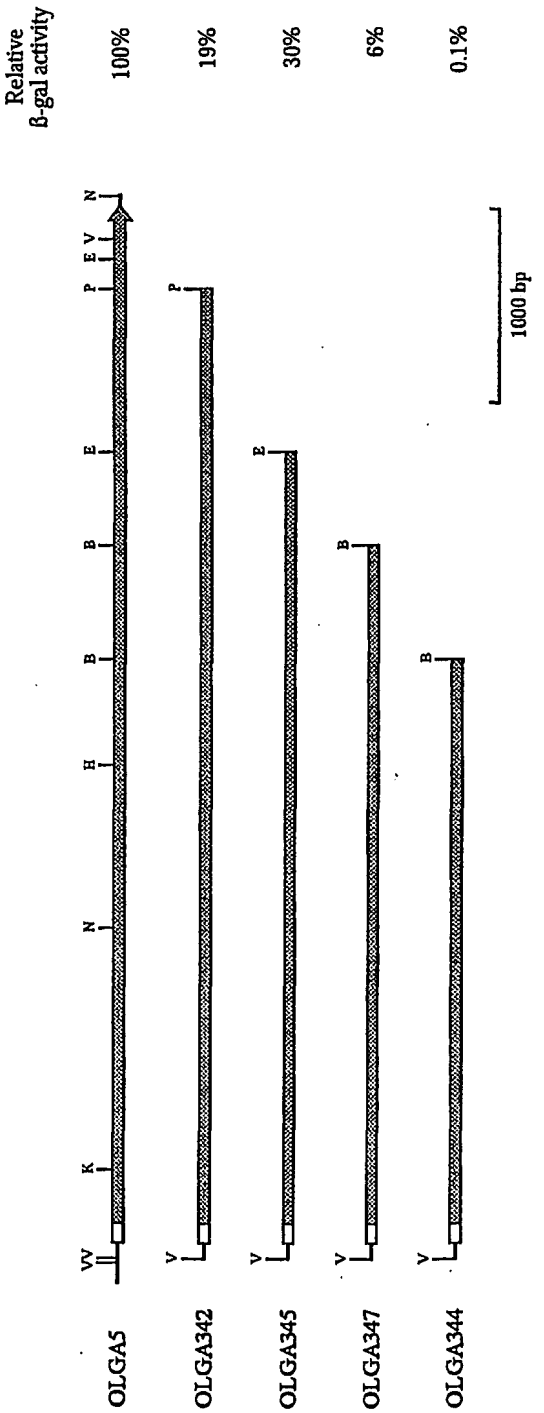

Relative
ß-gal activity
100%
19%
30%
6%
0.1%
OLGA5
OLGA342
OLGA345
OLGA347
OLGA344
1000 bp

Fig. 5

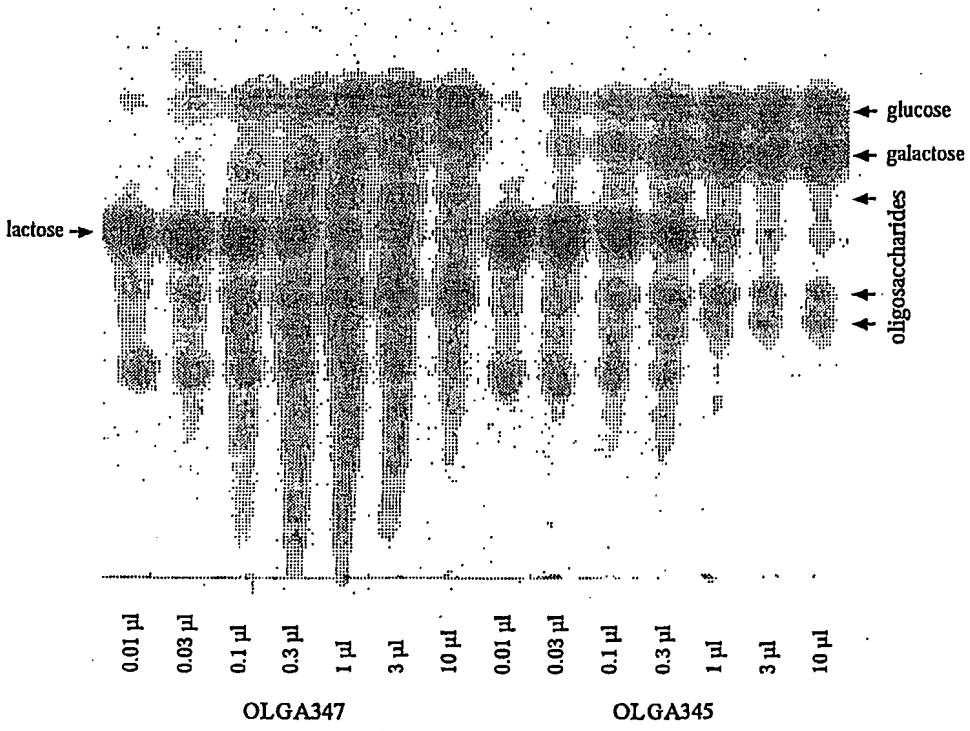
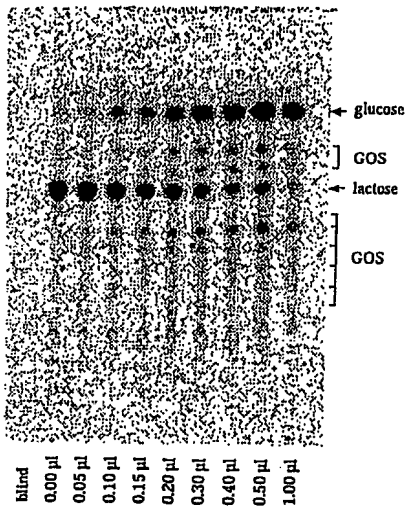

glucose
galactose
oligosaccharides
lactose
0.01 µl
0.03 µl
0.1 µl
0.3 µl
1 µl
3 µl
10 µl
0.01 µl
0.03 µl
0.1 µl
0.3 µl
1 µl
3 µl
10 µl
OLGA347
OLGA345

Fig. 6

(A)


glucose
GOS
lactose
GOS
blind
0.00 µl
0.05 µl
0.10 µl
0.15 µl
0.20 µl
0.30 µl
0.40 µl
0.50 µl
1.00 µl

(B)

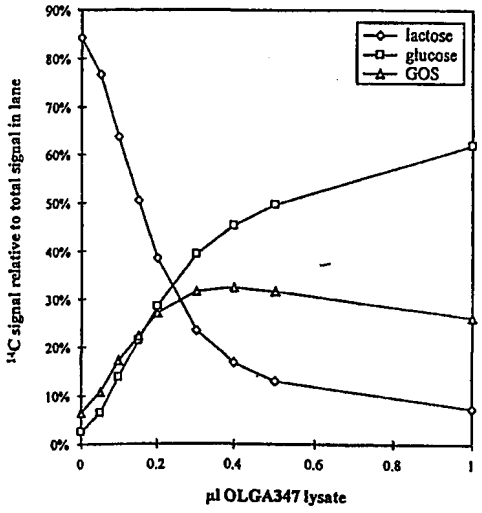

lactose
glucose
GOS
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
0.2
0.4
0.6
0.8
1
µl OLGA347 lysate
¹⁴C signal relative to total signal in lane

FIG. 7

(A) Reaction with 10% lactose.

	0 µl	0.1 µl	0.2 µl	0.4 µl	0.8 µl	1.5 µl	3 µl	6 µl
lactose	112.38	105.87	101.35	92.52	75.56	51.82	34.04	30.08
glucose	0	1.52	2.85	6.11	11.53	20.66	30.16	36.92
galactose	0	0.19	0.30	0.66	1.30	2.16	3.80	5.58

(B) Reaction with 20% lactose.

	0 µl	0.1 µl	0.2 µl	0.4 µl	0.8 µl	1.5 µl	3 µl	6 µl
lactose	235.65	217.58	205.30	177.70	137.27	93.78	66.24	61.69
glucose	0	2.95	6.48	13.93	29.57	45.99	61.06	73.06
galactose	0	0.34	0.48	0.78	1.96	3.07	4.87	6.95

(C) Reaction with 40% lactose.

	0 µl	0.1 µl	0.2 µl	0.4 µl	0.8 µl	1.5 µl	3 µl	6 µl
lactose	426.47	395.16	370.29	308.07	224.08	174.88	136.73	121.29
glucose	0	7.96	17.51	37.96	63.42	93.99	123.99	144.27
galactose	0	0.65	0.97	1.48	2.94	4.11	6.84	8.89

(D) Plot of reaction with 10% lactose.

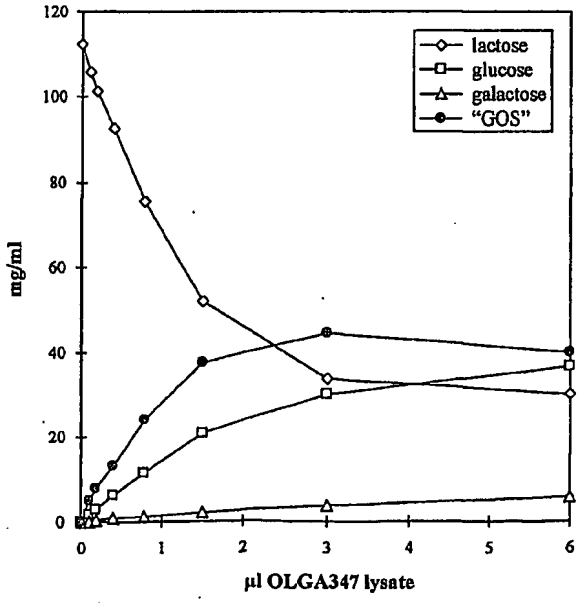


Fig. 8

SEQUENCE LISTING

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<151> 2000-05-26

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<170> PatentIn Ver. 2.1

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tcaccgtgtg gatatcgcat tggtgcgtat acactaacag caacagagcg gcgcggcagg 180

cgctcgtgga ttcaatgaag aaggaacgtt t atg gca gtt cgc aga ctt ggt 232
Met Ala Val Arg Arg Leu Gly
1 5

ggc cgc atc gtg gct ttc gcc gcc aca gtg gcc ttg tca ata ccg tta 280
Gly Arg Ile Val Ala Phe Ala Ala Thr Val Ala Leu Ser Ile Pro Leu
10 15 20

ggg ttg tta aca aat tca gcg tgg gcg gtc gag gac gcc acc cga tcc 328
Gly Leu Leu Thr Asn Ser Ala Trp Ala Val Glu Asp Ala Thr Arg Ser
25 30 35

gac tcc acc acg cag atg agc tcc acg ccg gag gtg gtc tac tcc agc 376
Asp Ser Thr Thr Gln Met Ser Ser Thr Pro Glu Val Val Tyr Ser Ser
40 45 50 55

gcc gtg gat tcc aag cag aat cgc acc tcg gat ttc gac gcc aac tgg 424
Ala Val Asp Ser Lys Gln Asn Arg Thr Ser Asp Phe Asp Ala Asn Trp
60 65 70

aag ttc atg ctg tcc gat tcc gtg cag gcg cag gat ccg gcg ttc gac 472
Lys Phe Met Leu Ser Asp Ser Val Gln Ala Gln Asp Pro Ala Phe Asp
75 80 85

gat tcg gcc tgg cag cag gtc gac ctg ccg cat gac tac agc atc acg 520
Asp Ser Ala Trp Gln Gln Val Asp Leu Pro His Asp Tyr Ser Ile Thr
90 95 100

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cag aag tat tcg cag agc aac gag gcc gaa agc gca tac ctt ccc ggc   568
Gln Lys Tyr Ser Gln Ser Asn Glu Ala Glu Ser Ala Tyr Leu Pro Gly
    105                 110                 115

ggc acc ggc tgg tac cgc aag tcc ttc acc atc gac cgg gac ctc gcc   616
Gly Thr Gly Trp Tyr Arg Lys Ser Phe Thr Ile Asp Arg Asp Leu Ala
120                 125                 130                 135

ggc aag cgc atc gcc atc aac ttc gac ggc gtg tac atg aac gcc acc   664
Gly Lys Arg Ile Ala Ile Asn Phe Asp Gly Val Tyr Met Asn Ala Thr
                140                 145                 150

gtc tgg ttc aac ggc gtc aag ctc ggc acc cat ccg tac ggc tac tcg   712
Val Trp Phe Asn Gly Val Lys Leu Gly Thr His Pro Tyr Gly Tyr Ser
            155                 160                 165

ccg ttc tcc ttc gac ctg acc ggc aac gcc aag ttc ggt ggg gag aac   760
Pro Phe Ser Phe Asp Leu Thr Gly Asn Ala Lys Phe Gly Gly Glu Asn
        170                 175                 180

acc atc gtc gtc aag gtc gag aac agg ctg ccg tcc agc cgc tgg tac   808
Thr Ile Val Val Lys Val Glu Asn Arg Leu Pro Ser Ser Arg Trp Tyr
    185                 190                 195

tcc ggc tcc ggc atc tac cgc gac gtc acc ctc acc gtc acc gac ggc   856
Ser Gly Ser Gly Ile Tyr Arg Asp Val Thr Leu Thr Val Thr Asp Gly
200                 205                 210                 215

gtg cac gtc ggc aat aac ggc gtg gcc atc aag acc ccg agc ctc gcc   904
Val His Val Gly Asn Asn Gly Val Ala Ile Lys Thr Pro Ser Leu Ala
                220                 225                 230

acc caa aac ggc ggc gac gtg acg atg aac ctc acc acc aag gtc gcc   952
Thr Gln Asn Gly Gly Asp Val Thr Met Asn Leu Thr Thr Lys Val Ala
            235                 240                 245

aac gac acc gag gcc gcg gcg aac atc acc ctc aag cag acc gtg ttc  1000
Asn Asp Thr Glu Ala Ala Ala Asn Ile Thr Leu Lys Gln Thr Val Phe
        250                 255                 260

ccc aag gga ggc aag acc gac gcc gcc atc ggc acc gtc acc acc gca  1048
Pro Lys Gly Gly Lys Thr Asp Ala Ala Ile Gly Thr Val Thr Thr Ala
    265                 270                 275

tcc aag tcc atc gcg gcc ggt gcc agc gcg gac gtg acc tcc acg atc  1096
Ser Lys Ser Ile Ala Ala Gly Ala Ser Ala Asp Val Thr Ser Thr Ile
280                 285                 290                 295

acc gcc gct tcg ccc aag ctg tgg agc atc aag aac ccg aac ctg tac  1144
Thr Ala Ala Ser Pro Lys Leu Trp Ser Ile Lys Asn Pro Asn Leu Tyr
                300                 305                 310

acc gtg cgc acc gaa gtg ctc aac ggc ggc aag gtg ctc gac act tac  1192
Thr Val Arg Thr Glu Val Leu Asn Gly Gly Lys Val Leu Asp Thr Tyr
            315                 320                 325

gac acc gaa tat ggc ttc cgc tgg acc ggc ttc gat gcg acc agc ggt  1240
Asp Thr Glu Tyr Gly Phe Arg Trp Thr Gly Phe Asp Ala Thr Ser Gly
        330                 335                 340

ttc tcg ctc aac ggc gag aaa gtc aag ctc aag ggc gtc tca atg cat  1288
Phe Ser Leu Asn Gly Glu Lys Val Lys Leu Lys Gly Val Ser Met His
    345                 350                 355
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cat gac cag gga tcg ctc ggc gcg gtc gcc aac cgc cgc gcc atc gag   1336
His Asp Gln Gly Ser Leu Gly Ala Val Ala Asn Arg Arg Ala Ile Glu
360                 365                 370                 375

cgc cag gtc gag att ctc cag aag atg ggc gtc aac tcg atc cgc acc   1384
Arg Gln Val Glu Ile Leu Gln Lys Met Gly Val Asn Ser Ile Arg Thr
                380                 385                 390

acg cac aac ccc gca gcc aag gcg ctg att gac gtc tgc aac gag aag   1432
Thr His Asn Pro Ala Ala Lys Ala Leu Ile Asp Val Cys Asn Glu Lys
            395                 400                 405

ggc gtc ctc gtg gtc gaa gag gtc ttc gac atg tgg aac cgg tcg aag   1480
Gly Val Leu Val Val Glu Glu Val Phe Asp Met Trp Asn Arg Ser Lys
        410                 415                 420

aac ggc aac acc gag gat tac ggc aag tgg ttc ggc cag gcc atc gcc   1528
Asn Gly Asn Thr Glu Asp Tyr Gly Lys Trp Phe Gly Gln Ala Ile Ala
    425                 430                 435

ggt gac aac gcc gtc ctg ggt ggc gac aag gac gag acc tgg gcc aag   1576
Gly Asp Asn Ala Val Leu Gly Gly Asp Lys Asp Glu Thr Trp Ala Lys
440                 445                 450                 455

ttc gac ctg acc agc acc atc aac cgt gac agg aac gcc ccg tcc gtc   1624
Phe Asp Leu Thr Ser Thr Ile Asn Arg Asp Arg Asn Ala Pro Ser Val
                460                 465                 470

atc atg tgg tcg ctc ggc aac gag atg atg gaa ggc atc agc ggc agc   1672
Ile Met Trp Ser Leu Gly Asn Glu Met Met Glu Gly Ile Ser Gly Ser
            475                 480                 485

gtc tcg ggc ttc ccg gct acc tcc gcc aag ctg gtc gca tgg acg aag   1720
Val Ser Gly Phe Pro Ala Thr Ser Ala Lys Leu Val Ala Trp Thr Lys
        490                 495                 500

gcc gcg gac agc acc cgc ccg atg acc tac ggc gac aac aag atc aag   1768
Ala Ala Asp Ser Thr Arg Pro Met Thr Tyr Gly Asp Asn Lys Ile Lys
    505                 510                 515

gcc aac tgg aac gag tcg aac acc atg ggc gac aac ctg acc gcc aac   1816
Ala Asn Trp Asn Glu Ser Asn Thr Met Gly Asp Asn Leu Thr Ala Asn
520                 525                 530                 535

ggc ggc gtg gtc ggc acc aac tac tcc gac ggc gcg aac tac gac aag   1864
Gly Gly Val Val Gly Thr Asn Tyr Ser Asp Gly Ala Asn Tyr Asp Lys
                540                 545                 550

atc cgc acg acc cac ccc tca tgg gcc atc tat ggt tcc gag acg gcg   1912
Ile Arg Thr Thr His Pro Ser Trp Ala Ile Tyr Gly Ser Glu Thr Ala
            555                 560                 565

tcc gcc atc aac agc cga ggc atc tac aac cgc acc acc ggc ggc gcc   1960
Ser Ala Ile Asn Ser Arg Gly Ile Tyr Asn Arg Thr Thr Gly Gly Ala
        570                 575                 580

cag tca agc gac aag cag ctg acc agc tat gac aat tcc gca gtc ggc   2008
Gln Ser Ser Asp Lys Gln Leu Thr Ser Tyr Asp Asn Ser Ala Val Gly
    585                 590                 595

tgg ggc gcc gtc gcc agc tcc gcc tgg tac gac gtg gtc cag cgc gat   2056
Trp Gly Ala Val Ala Ser Ser Ala Trp Tyr Asp Val Val Gln Arg Asp
600                 605                 610                 615
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ttc gtc gcc ggc aca tac gtg tgg acc ggc ttc gac tac ctc ggc gaa   2104
Phe Val Ala Gly Thr Tyr Val Trp Thr Gly Phe Asp Tyr Leu Gly Glu
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ccc acc ccg tgg aac ggc acc ggc tcc ggc gcc gtg ggc tcc ttg gcc   2152
Pro Thr Pro Trp Asn Gly Thr Gly Ser Gly Ala Val Gly Ser Leu Ala
            635                 640                 645

gtc gcc gaa gaa ctc gta ctt cgg cat cgt cga cac cgc agg ctt ccc   2200
Val Ala Glu Glu Leu Val Leu Arg His Arg Arg His Arg Arg Leu Pro
        650                 655                 660

gaa gac acc tat tac ttc tat cag agc cag tgg aac gac gac gtg cac   2248
Glu Asp Thr Tyr Tyr Phe Tyr Gln Ser Gln Trp Asn Asp Asp Val His
    665                 670                 675

acg ctg cac atc ctc ccc gca tgg aac gag aac gtc gtc gcc aag ggc   2296
Thr Leu His Ile Leu Pro Ala Trp Asn Glu Asn Val Val Ala Lys Gly
680                 685                 690                 695

tcc ggc aac aac gtg ccg gtc gtc gtc tac acc gac gcg gcc aag gtc   2344
Ser Gly Asn Asn Val Pro Val Val Val Tyr Thr Asp Ala Ala Lys Val
                700                 705                 710

aag ctg tac ttc aca ccg aag ggc agt acc gaa aag cga ctg atc gga   2392
Lys Leu Tyr Phe Thr Pro Lys Gly Ser Thr Glu Lys Arg Leu Ile Gly
            715                 720                 725

gag aag tcc ttc acc aag aag acc acc gcg gcc gga tac acc tat cag   2440
Glu Lys Ser Phe Thr Lys Lys Thr Thr Ala Ala Gly Tyr Thr Tyr Gln
        730                 735                 740

gtc tac gag ggc tcc gac aag gac tcc acc gcc cac aag aac atg tac   2488
Val Tyr Glu Gly Ser Asp Lys Asp Ser Thr Ala His Lys Asn Met Tyr
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ctg acc tgg aac gtg ccg tgg gcc gag ggc acc atc tcc gcc gaa gca   2536
Leu Thr Trp Asn Val Pro Trp Ala Glu Gly Thr Ile Ser Ala Glu Ala
760                 765                 770                 775

tac gac gag aac aac agg ctg atc ccc gag ggg tcc acc gag ggc aac   2584
Tyr Asp Glu Asn Asn Arg Leu Ile Pro Glu Gly Ser Thr Glu Gly Asn
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gcg tcg gtg acc acc acc ggc aag gcc gcg aag ctt aaa gcc gat gcc   2632
Ala Ser Val Thr Thr Thr Gly Lys Ala Ala Lys Leu Lys Ala Asp Ala
            795                 800                 805

gac cgc aag acg atc acc gcg gac ggc aag gac ctg tcg tac atc gag   2680
Asp Arg Lys Thr Ile Thr Ala Asp Gly Lys Asp Leu Ser Tyr Ile Glu
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gtc gac gtg acc gac gcc aac ggc cat atc gtc ccc gat gcc gcc aac   2728
Val Asp Val Thr Asp Ala Asn Gly His Ile Val Pro Asp Ala Ala Asn
    825                 830                 835

cgc gtc acc ttc gac gtc aag ggc gcc ggc aaa ctg gtc ggc gtc gac   2776
Arg Val Thr Phe Asp Val Lys Gly Ala Gly Lys Leu Val Gly Val Asp
840                 845                 850                 855

aac ggc agc tcg ccg gat cac gac tcc tat cag gcc gac aac cgc aag   2824
Asn Gly Ser Ser Pro Asp His Asp Ser Tyr Gln Ala Asp Asn Arg Lys
                860                 865                 870
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Ala Phe Ser Gly Lys Val Leu Ala Ile Val Gln Ser Thr Lys Glu Ala
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ggc gag atc acc gtc acc gcc aag gcc gac ggt ctg caa tca tcc aca   2920
Gly Glu Ile Thr Val Thr Ala Lys Ala Asp Gly Leu Gln Ser Ser Thr
        890                 895                 900

gtg aag atc gcc acc acc gcc gtc ccc ggc acc agc acc gag aag acg   2968
Val Lys Ile Ala Thr Thr Ala Val Pro Gly Thr Ser Thr Glu Lys Thr
    905                 910                 915

gtc cgc agc ttc tac tac tcg cgc aac tac tac gtc aag acc ggc aac   3016
Val Arg Ser Phe Tyr Tyr Ser Arg Asn Tyr Tyr Val Lys Thr Gly Asn
920                 925                 930                 935

aag ccg att ctg ccg agt gat gtc gag gtg cgc tac tcc gac ggc acg   3064
Lys Pro Ile Leu Pro Ser Asp Val Glu Val Arg Tyr Ser Asp Gly Thr
                940                 945                 950

tcg gac cgt cag aac gtc aca tgg gac gca gtc agc gac gac cag atc   3112
Ser Asp Arg Gln Asn Val Thr Trp Asp Ala Val Ser Asp Asp Gln Ile
            955                 960                 965

gcc aag gcc ggt tcg ttc agc gtg gcc ggc acg gtc gcc ggg cag aag   3160
Ala Lys Ala Gly Ser Phe Ser Val Ala Gly Thr Val Ala Gly Gln Lys
        970                 975                 980

atc tcc gtg cgc gtg acg atg atc gac gag atc ggt gcg ctg ctc aac   3208
Ile Ser Val Arg Val Thr Met Ile Asp Glu Ile Gly Ala Leu Leu Asn
    985                 990                 995

tat tcg gcc agc aca ccg gtc ggc acg ccc gcc gtg ctg cct ggc tcg   3256
Tyr Ser Ala Ser Thr Pro Val Gly Thr Pro Ala Val Leu Pro Gly Ser
1000                1005                1010                1015

cgt ccg gcc gtg ctg ccc gac ggc acc gtg acc agc gcg aac ttc gcc   3304
Arg Pro Ala Val Leu Pro Asp Gly Thr Val Thr Ser Ala Asn Phe Ala
                1020                1025                1030

gtc cac tgg acc aag ccc gcc gac acc gtg tac aac acg gcc ggc acc   3352
Val His Trp Thr Lys Pro Ala Asp Thr Val Tyr Asn Thr Ala Gly Thr
            1035                1040                1045

gtc aag gtc ccc ggc acc gcc acc gtc ttc ggc aag gag ttc aag gtc   3400
Val Lys Val Pro Gly Thr Ala Thr Val Phe Gly Lys Glu Phe Lys Val
        1050                1055                1060

acc gcg acg att cgc gtg cag cgg tcg cag gtc acc atc ggc agc agc   3448
Thr Ala Thr Ile Arg Val Gln Arg Ser Gln Val Thr Ile Gly Ser Ser
    1065                1070                1075

gtc tcc ggc aat gcg ctg cgc ctg act cag aac atc ccc gcc gac aag   3496
Val Ser Gly Asn Ala Leu Arg Leu Thr Gln Asn Ile Pro Ala Asp Lys
1080                1085                1090                1095

cag tcc gac acg ctg gac gcc atc aag gac ggc tcc acg acc gtc gac   3544
Gln Ser Asp Thr Leu Asp Ala Ile Lys Asp Gly Ser Thr Thr Val Asp
                1100                1105                1110

gcc aat acc ggc ggc ggc gcg aac ccg tca gca tgg acc aac tgg gcg   3592
Ala Asn Thr Gly Gly Gly Ala Asn Pro Ser Ala Trp Thr Asn Trp Ala
            1115                1120                1125
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acc gag cag cag ctc ggc cag att gtc atg tac ttc ttc cgc gac agc  3688
Thr Glu Gln Gln Leu Gly Gln Ile Val Met Tyr Phe Phe Arg Asp Ser
   1145                1150                1155

aac gcg gtg agg ttc ccc gac gcc ggc aag acg aag atc cag atc tcc  3736
Asn Ala Val Arg Phe Pro Asp Ala Gly Lys Thr Lys Ile Gln Ile Ser
1160                1165                1170                1175

gcg gac ggc aag aac tgg acg gat ctc gct gcc acg gag acc atc gcg  3784
Ala Asp Gly Lys Asn Trp Thr Asp Leu Ala Ala Thr Glu Thr Ile Ala
                1180                1185                1190

gcc cag gag tcg tcc gac cga gtc aag ccg tac acc tat gac ttc gct  3832
Ala Gln Glu Ser Ser Asp Arg Val Lys Pro Tyr Thr Tyr Asp Phe Ala
          1195                1200                1205

ccg gtg gga gcc acg ttc gtc aag gtc acg gtc acc aac gcc gac acc  3880
Pro Val Gly Ala Thr Phe Val Lys Val Thr Val Thr Asn Ala Asp Thr
      1210                1215                1220

aca acc ccc agc ggc gtg gtc tgc gcc ggc ctg acc gag atc gag ctg  3928
Thr Thr Pro Ser Gly Val Val Cys Ala Gly Leu Thr Glu Ile Glu Leu
   1225                1230                1235

aag acc gcg acc agc aag ttc gtc acg aac acg tcc gcc gcg ctc tcg  3976
Lys Thr Ala Thr Ser Lys Phe Val Thr Asn Thr Ser Ala Ala Leu Ser
1240                1245                1250                1255

tcg ctg aca gtg aac ggc acg aag gtc tcc gac tcc gtg ctc gcc gcc  4024
Ser Leu Thr Val Asn Gly Thr Lys Val Ser Asp Ser Val Leu Ala Ala
                1260                1265                1270

ggc tcc tac aac acg ccc gcg atc atc gcg gac gtc aaa gcc gag ggc  4072
Gly Ser Tyr Asn Thr Pro Ala Ile Ile Ala Asp Val Lys Ala Glu Gly
          1275                1280                1285

gaa ggc aac gcc agc gtc acc gtg ctg ccc gcg cac gac aac gtg atc  4120
Glu Gly Asn Ala Ser Val Thr Val Leu Pro Ala His Asp Asn Val Ile
      1290                1295                1300

cgc gtg atc acc gag tcc gag gac cac gtc acg cgc aag acc ttc acc  4168
Arg Val Ile Thr Glu Ser Glu Asp His Val Thr Arg Lys Thr Phe Thr
   1305                1310                1315

atc aac ctg ggc acg gag cag gaa ttc ccc gca gac tcc gat gaa cgc  4216
Ile Asn Leu Gly Thr Glu Gln Glu Phe Pro Ala Asp Ser Asp Glu Arg
1320                1325                1330                1335

gac tac ccg gcc gcc gac atg acg gtc acc gtg ggc agc gaa cag acg  4264
Asp Tyr Pro Ala Ala Asp Met Thr Val Thr Val Gly Ser Glu Gln Thr
                1340                1345                1350

tcc ggc acc gcg acc gaa ggc ccg aag aaa ttc gcg gtc gac ggc aac  4312
Ser Gly Thr Ala Thr Glu Gly Pro Lys Lys Phe Ala Val Asp Gly Asn
          1355                1360                1365

acc agc acg tac tgg cat tcc aac tgg acg ccc acc acc gtg aac gac  4360
Thr Ser Thr Tyr Trp His Ser Asn Trp Thr Pro Thr Thr Val Asn Asp
      1370                1375                1380
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ctg tgg atc gcc ttc gag ctc cag aaa ccc acc aag ctc gac gcg ctg   4408
Leu Trp Ile Ala Phe Glu Leu Gln Lys Pro Thr Lys Leu Asp Ala Leu
   1385                1390                1395

cgc tac ctg ccg cgc ccc gcg ggc agc aag aac ggc tcc gtc acc gaa   4456
Arg Tyr Leu Pro Arg Pro Ala Gly Ser Lys Asn Gly Ser Val Thr Glu
1400                1405                1410                1415

tac aag gtt cag gtc agc gat gac ggc acc aac tgg acc gac gcg ggc   4504
Tyr Lys Val Gln Val Ser Asp Asp Gly Thr Asn Trp Thr Asp Ala Gly
                1420                1425                1430

tcc ggc aca tgg acc acc gat tac ggc tgg aag ctc gcc gag ttc aat   4552
Ser Gly Thr Trp Thr Thr Asp Tyr Gly Trp Lys Leu Ala Glu Phe Asn
            1435                1440                1445

cag ccg gtg acc acc aag cac gtg cgg ctc aag gcc gtc cac acc tat   4600
Gln Pro Val Thr Thr Lys His Val Arg Leu Lys Ala Val His Thr Tyr
        1450                1455                1460

gcg gat tcc ggc aac gac aag ttc atg tcc gcc tcc gaa atc cgc ctg   4648
Ala Asp Ser Gly Asn Asp Lys Phe Met Ser Ala Ser Glu Ile Arg Leu
   1465                1470                1475

cgc aag gcc gtc gac acc acc gac atc agc ggc gcg acc gtg acc gtg   4696
Arg Lys Ala Val Asp Thr Thr Asp Ile Ser Gly Ala Thr Val Thr Val
1480                1485                1490                1495

ccc gcc aag ctg acc gtc gac cgg gtg gac gcc gac cat ccc gcc acc   4744
Pro Ala Lys Leu Thr Val Asp Arg Val Asp Ala Asp His Pro Ala Thr
                1500                1505                1510

ttc gcc acg aag gac gtg acg gtg acg ttg ggc gac gcc acg ctg cgc   4792
Phe Ala Thr Lys Asp Val Thr Val Thr Leu Gly Asp Ala Thr Leu Arg
            1515                1520                1525

tac ggc gtg gac tac ctg ctc gac tac gcg ggc aac acc gcc gtc ggc   4840
Tyr Gly Val Asp Tyr Leu Leu Asp Tyr Ala Gly Asn Thr Ala Val Gly
        1530                1535                1540

aag gcc acg gtg acc gtg cgc ggc atc gac aag tac tcc ggc acc gtc   4888
Lys Ala Thr Val Thr Val Arg Gly Ile Asp Lys Tyr Ser Gly Thr Val
   1545                1550                1555

gcc aag acg ttc acc atc gaa ctg aag aac gcc ccg gcg ccg gaa ccg   4936
Ala Lys Thr Phe Thr Ile Glu Leu Lys Asn Ala Pro Ala Pro Glu Pro
1560                1565                1570                1575

acg ctg acc tcg gtg agc gtc aag acc aag cct tcc aag ctg acc tat   4984
Thr Leu Thr Ser Val Ser Val Lys Thr Lys Pro Ser Lys Leu Thr Tyr
                1580                1585                1590

gtg gtc ggc gac gcg ttc gac ccg gca gga ctg gtg ctg cag cac gac   5032
Val Val Gly Asp Ala Phe Asp Pro Ala Gly Leu Val Leu Gln His Asp
            1595                1600                1605

aga cag gcc gat cgc ccc cca cag cca ctt gtt gga gaa cag gcc gac   5080
Arg Gln Ala Asp Arg Pro Pro Gln Pro Leu Val Gly Glu Gln Ala Asp
        1610                1615                1620

gaa cgc gga ctg acg tgc gga acg cga tgc gat cgc gtt gaa cag ctg   5128
Glu Arg Gly Leu Thr Cys Gly Thr Arg Cys Asp Arg Val Glu Gln Leu
   1625                1630                1635
```

```
cgc aaa cac gag aat cgt gaa gcc cat cgt acg ggc ctc gat cat ctg   5176
Arg Lys His Glu Asn Arg Glu Ala His Arg Thr Gly Leu Asp His Leu
1640                1645                1650                1655

gaa ttc gtg ggt gcc gcc gat gga gcg gtc ggt gaa cag gcc acc ttc   5224
Glu Phe Val Gly Ala Ala Asp Gly Ala Val Gly Glu Gln Ala Thr Phe
                  1660                1665                1670

aag gtg cat gtc cat gcc gat caa ggt gac ggc cgc cat gat gat gcc   5272
Lys Val His Val His Ala Asp Gln Gly Asp Gly Arg His Asp Asp Ala
             1675                1680                1685

gat gaa cgc gat atc gat cca cat gtc cct gtc gat cac gcg gtc ggt   5320
Asp Glu Arg Asp Ile Asp Pro His Val Pro Val Asp His Ala Val Gly
        1690                1695                1700

gag ctt gcg cgg gct gcg tgc cat cac gtc atc ggt ctg cgg gtc gac   5368
Glu Leu Ala Arg Ala Ala Cys His His Val Ile Gly Leu Arg Val Asp
   1705                1710                1715

acc cat cgc ctc aag gca tcc ggc ttc cag atc ccc gcc gac gac atg   5416
Thr His Arg Leu Lys Ala Ser Gly Phe Gln Ile Pro Ala Asp Asp Met
1720                1725                1730                1735

gcc gag atc gac cgc atc acc ggc ttc cac cgc ttc gag cgc cac gtc   5464
Ala Glu Ile Asp Arg Ile Thr Gly Phe His Arg Phe Glu Arg His Val
                  1740                1745                1750

ggc tga cgtgattggg cttccccgct gtctggtgcc ggctcgcga               5509
Gly
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<210> 2
<211> 1752
<212> PRT
<213> Bifidobacterium bifidum

<400> 2

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Met Ala Val Arg Arg Leu Gly Gly Arg Ile Val Ala Phe Ala Ala Thr
  1               5                  10                  15
Val Ala Leu Ser Ile Pro Leu Gly Leu Leu Thr Asn Ser Ala Trp Ala
             20                  25                  30
Val Glu Asp Ala Thr Arg Ser Asp Ser Thr Thr Gln Met Ser Ser Thr
         35                  40                  45
Pro Glu Val Val Tyr Ser Ser Ala Val Asp Ser Lys Gln Asn Arg Thr
     50                  55                  60
Ser Asp Phe Asp Ala Asn Trp Lys Phe Met Leu Ser Asp Ser Val Gln
 65                  70                  75                  80
Ala Gln Asp Pro Ala Phe Asp Asp Ser Ala Trp Gln Gln Val Asp Leu
                 85                  90                  95
Pro His Asp Tyr Ser Ile Thr Gln Lys Tyr Ser Gln Ser Asn Glu Ala
            100                 105                 110
Glu Ser Ala Tyr Leu Pro Gly Gly Thr Gly Trp Tyr Arg Lys Ser Phe
        115                 120                 125
Thr Ile Asp Arg Asp Leu Ala Gly Lys Arg Ile Ala Ile Asn Phe Asp
    130                 135                 140
Gly Val Tyr Met Asn Ala Thr Val Trp Phe Asn Gly Val Lys Leu Gly
145                 150                 155                 160
Thr His Pro Tyr Gly Tyr Ser Pro Phe Ser Phe Asp Leu Thr Gly Asn
                165                 170                 175
Ala Lys Phe Gly Gly Glu Asn Thr Ile Val Val Lys Val Glu Asn Arg
            180                 185                 190
Leu Pro Ser Ser Arg Trp Tyr Ser Gly Ser Gly Ile Tyr Arg Asp Val
        195                 200                 205
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Thr Leu Thr Val Thr Asp Gly Val His Val Gly Asn Asn Gly Val Ala
    210                 215                 220
Ile Lys Thr Pro Ser Leu Ala Thr Gln Asn Gly Gly Asp Val Thr Met
225                 230                 235                 240
Asn Leu Thr Thr Lys Val Ala Asn Asp Thr Glu Ala Ala Ala Asn Ile
                245                 250                 255
Thr Leu Lys Gln Thr Val Phe Pro Lys Gly Gly Lys Thr Asp Ala Ala
            260                 265                 270
Ile Gly Thr Val Thr Thr Ala Ser Lys Ser Ile Ala Ala Gly Ala Ser
        275                 280                 285
Ala Asp Val Thr Ser Thr Ile Thr Ala Ala Ser Pro Lys Leu Trp Ser
    290                 295                 300
Ile Lys Asn Pro Asn Leu Tyr Thr Val Arg Thr Glu Val Leu Asn Gly
305                 310                 315                 320
Gly Lys Val Leu Asp Thr Tyr Asp Thr Glu Tyr Gly Phe Arg Trp Thr
                325                 330                 335
Gly Phe Asp Ala Thr Ser Gly Phe Ser Leu Asn Gly Glu Lys Val Lys
            340                 345                 350
Leu Lys Gly Val Ser Met His His Asp Gln Gly Ser Leu Gly Ala Val
        355                 360                 365
Ala Asn Arg Arg Ala Ile Glu Arg Gln Val Glu Ile Leu Gln Lys Met
    370                 375                 380
Gly Val Asn Ser Ile Arg Thr Thr His Asn Pro Ala Ala Lys Ala Leu
385                 390                 395                 400
Ile Asp Val Cys Asn Glu Lys Gly Val Leu Val Val Glu Glu Val Phe
                405                 410                 415
Asp Met Trp Asn Arg Ser Lys Asn Gly Asn Thr Glu Asp Tyr Gly Lys
            420                 425                 430
Trp Phe Gly Gln Ala Ile Ala Gly Asp Asn Ala Val Leu Gly Gly Asp
        435                 440                 445
Lys Asp Glu Thr Trp Ala Lys Phe Asp Leu Thr Ser Thr Ile Asn Arg
    450                 455                 460
Asp Arg Asn Ala Pro Ser Val Ile Met Trp Ser Leu Gly Asn Glu Met
465                 470                 475                 480
Met Glu Gly Ile Ser Gly Ser Val Ser Gly Phe Pro Ala Thr Ser Ala
                485                 490                 495
Lys Leu Val Ala Trp Thr Lys Ala Ala Asp Ser Thr Arg Pro Met Thr
            500                 505                 510
Tyr Gly Asp Asn Lys Ile Lys Ala Asn Trp Asn Glu Ser Asn Thr Met
        515                 520                 525
Gly Asp Asn Leu Thr Ala Asn Gly Gly Val Val Gly Thr Asn Tyr Ser
    530                 535                 540
Asp Gly Ala Asn Tyr Asp Lys Ile Arg Thr Thr His Pro Ser Trp Ala
545                 550                 555                 560
Ile Tyr Gly Ser Glu Thr Ala Ser Ala Ile Asn Ser Arg Gly Ile Tyr
                565                 570                 575
Asn Arg Thr Thr Gly Gly Ala Gln Ser Ser Asp Lys Gln Leu Thr Ser
            580                 585                 590
Tyr Asp Asn Ser Ala Val Gly Trp Gly Ala Val Ala Ser Ser Ala Trp
        595                 600                 605
Tyr Asp Val Val Gln Arg Asp Phe Val Ala Gly Thr Tyr Val Trp Thr
    610                 615                 620
Gly Phe Asp Tyr Leu Gly Glu Pro Thr Pro Trp Asn Gly Thr Gly Ser
625                 630                 635                 640
Gly Ala Val Gly Ser Leu Ala Val Ala Glu Glu Leu Val Leu Arg His
                645                 650                 655
Arg Arg His Arg Arg Leu Pro Glu Asp Thr Tyr Tyr Phe Tyr Gln Ser
            660                 665                 670
Gln Trp Asn Asp Asp Val His Thr Leu His Ile Leu Pro Ala Trp Asn
        675                 680                 685
Glu Asn Val Val Ala Lys Gly Ser Gly Asn Asn Val Pro Val Val Val
    690                 695                 700
Tyr Thr Asp Ala Ala Lys Val Lys Leu Tyr Phe Thr Pro Lys Gly Ser
705                 710                 715                 720
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Thr Glu Lys Arg Leu Ile Gly Glu Lys Ser Phe Thr Lys Lys Thr Thr
                725                 730                 735
Ala Ala Gly Tyr Thr Tyr Gln Val Tyr Glu Gly Ser Asp Lys Asp Ser
            740                 745                 750
Thr Ala His Lys Asn Met Tyr Leu Thr Trp Asn Val Pro Trp Ala Glu
        755                 760                 765
Gly Thr Ile Ser Ala Glu Ala Tyr Asp Glu Asn Asn Arg Leu Ile Pro
    770                 775                 780
Glu Gly Ser Thr Glu Gly Asn Ala Ser Val Thr Thr Thr Gly Lys Ala
785                 790                 795                 800
Ala Lys Leu Lys Ala Asp Ala Asp Arg Lys Thr Ile Thr Ala Asp Gly
                805                 810                 815
Lys Asp Leu Ser Tyr Ile Glu Val Asp Val Thr Asp Ala Asn Gly His
            820                 825                 830
Ile Val Pro Asp Ala Ala Asn Arg Val Thr Phe Asp Val Lys Gly Ala
        835                 840                 845
Gly Lys Leu Val Gly Val Asp Asn Gly Ser Ser Pro Asp His Asp Ser
    850                 855                 860
Tyr Gln Ala Asp Asn Arg Lys Ala Phe Ser Gly Lys Val Leu Ala Ile
865                 870                 875                 880
Val Gln Ser Thr Lys Glu Ala Gly Glu Ile Thr Val Thr Ala Lys Ala
                885                 890                 895
Asp Gly Leu Gln Ser Ser Thr Val Lys Ile Ala Thr Thr Ala Val Pro
            900                 905                 910
Gly Thr Ser Thr Glu Lys Thr Val Arg Ser Phe Tyr Tyr Ser Arg Asn
        915                 920                 925
Tyr Tyr Val Lys Thr Gly Asn Lys Pro Ile Leu Pro Ser Asp Val Glu
    930                 935                 940
Val Arg Tyr Ser Asp Gly Thr Ser Asp Arg Gln Asn Val Thr Trp Asp
945                 950                 955                 960
Ala Val Ser Asp Asp Gln Ile Ala Lys Ala Gly Ser Phe Ser Val Ala
                965                 970                 975
Gly Thr Val Ala Gly Gln Lys Ile Ser Val Arg Val Thr Met Ile Asp
            980                 985                 990
Glu Ile Gly Ala Leu Leu Asn Tyr Ser Ala Ser Thr Pro Val Gly Thr
        995                1000                1005
Pro Ala Val Leu Pro Gly Ser Arg Pro Ala Val Leu Pro Asp Gly Thr
   1010                1015                1020
Val Thr Ser Ala Asn Phe Ala Val His Trp Thr Lys Pro Ala Asp Thr
1025                1030                1035                1040
Val Tyr Asn Thr Ala Gly Thr Val Lys Val Pro Gly Thr Ala Thr Val
                1045                1050                1055
Phe Gly Lys Glu Phe Lys Val Thr Ala Thr Ile Arg Val Gln Arg Ser
            1060                1065                1070
Gln Val Thr Ile Gly Ser Ser Val Ser Gly Asn Ala Leu Arg Leu Thr
        1075                1080                1085
Gln Asn Ile Pro Ala Asp Lys Gln Ser Asp Thr Leu Asp Ala Ile Lys
   1090                1095                1100
Asp Gly Ser Thr Thr Val Asp Ala Asn Thr Gly Gly Gly Ala Asn Pro
1105                1110                1115                1120
Ser Ala Trp Thr Asn Trp Ala Tyr Ser Lys Ala Gly His Asn Thr Ala
                1125                1130                1135
Glu Ile Thr Phe Glu Tyr Ala Thr Glu Gln Gln Leu Gly Gln Ile Val
            1140                1145                1150
Met Tyr Phe Phe Arg Asp Ser Asn Ala Val Arg Phe Pro Asp Ala Gly
        1155                1160                1165
Lys Thr Lys Ile Gln Ile Ser Ala Asp Gly Lys Asn Trp Thr Asp Leu
   1170                1175                1180
Ala Ala Thr Glu Thr Ile Ala Ala Gln Glu Ser Ser Asp Arg Val Lys
1185                1190                1195                1200
Pro Tyr Thr Tyr Asp Phe Ala Pro Val Gly Ala Thr Phe Val Lys Val
                1205                1210                1215
Thr Val Thr Asn Ala Asp Thr Thr Thr Pro Ser Gly Val Val Cys Ala
            1220                1225                1230
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Gly Leu Thr Glu Ile Glu Leu Lys Thr Ala Thr Ser Lys Phe Val Thr
        1235                1240                1245
Asn Thr Ser Ala Ala Leu Ser Ser Leu Thr Val Asn Gly Thr Lys Val
    1250                1255                1260
Ser Asp Ser Val Leu Ala Ala Gly Ser Tyr Asn Thr Pro Ala Ile Ile
1265                1270                1275                1280
Ala Asp Val Lys Ala Glu Gly Glu Gly Asn Ala Ser Val Thr Val Leu
                1285                1290                1295
Pro Ala His Asp Asn Val Ile Arg Val Ile Thr Glu Ser Glu Asp His
            1300                1305                1310
Val Thr Arg Lys Thr Phe Thr Ile Asn Leu Gly Thr Glu Gln Glu Phe
        1315                1320                1325
Pro Ala Asp Ser Asp Glu Arg Asp Tyr Pro Ala Ala Asp Met Thr Val
    1330                1335                1340
Thr Val Gly Ser Glu Gln Thr Ser Gly Thr Ala Thr Glu Gly Pro Lys
1345                1350                1355                1360
Lys Phe Ala Val Asp Gly Asn Thr Ser Thr Tyr Trp His Ser Asn Trp
                1365                1370                1375
Thr Pro Thr Thr Val Asn Asp Leu Trp Ile Ala Phe Glu Leu Gln Lys
            1380                1385                1390
Pro Thr Lys Leu Asp Ala Leu Arg Tyr Leu Pro Arg Pro Ala Gly Ser
        1395                1400                1405
Lys Asn Gly Ser Val Thr Glu Tyr Lys Val Gln Val Ser Asp Asp Gly
    1410                1415                1420
Thr Asn Trp Thr Asp Ala Gly Ser Gly Thr Trp Thr Thr Asp Tyr Gly
1425                1430                1435                1440
Trp Lys Leu Ala Glu Phe Asn Gln Pro Val Thr Thr Lys His Val Arg
                1445                1450                1455
Leu Lys Ala Val His Thr Tyr Ala Asp Ser Gly Asn Asp Lys Phe Met
            1460                1465                1470
Ser Ala Ser Glu Ile Arg Leu Arg Lys Ala Val Asp Thr Thr Asp Ile
        1475                1480                1485
Ser Gly Ala Thr Val Thr Val Pro Ala Lys Leu Thr Val Asp Arg Val
    1490                1495                1500
Asp Ala Asp His Pro Ala Thr Phe Ala Thr Lys Asp Val Thr Val Thr
1505                1510                1515                1520
Leu Gly Asp Ala Thr Leu Arg Tyr Gly Val Asp Tyr Leu Leu Asp Tyr
                1525                1530                1535
Ala Gly Asn Thr Ala Val Gly Lys Ala Thr Val Thr Val Arg Gly Ile
            1540                1545                1550
Asp Lys Tyr Ser Gly Thr Val Ala Lys Thr Phe Thr Ile Glu Leu Lys
        1555                1560                1565
Asn Ala Pro Ala Pro Glu Pro Thr Leu Thr Ser Val Ser Val Lys Thr
    1570                1575                1580
Lys Pro Ser Lys Leu Thr Tyr Val Val Gly Asp Ala Phe Asp Pro Ala
1585                1590                1595                1600
Gly Leu Val Leu Gln His Asp Arg Gln Ala Asp Arg Pro Pro Gln Pro
                1605                1610                1615
Leu Val Gly Glu Gln Ala Asp Glu Arg Gly Leu Thr Cys Gly Thr Arg
            1620                1625                1630
Cys Asp Arg Val Glu Gln Leu Arg Lys His Glu Asn Arg Glu Ala His
        1635                1640                1645
Arg Thr Gly Leu Asp His Leu Glu Phe Val Gly Ala Ala Asp Gly Ala
    1650                1655                1660
Val Gly Glu Gln Ala Thr Phe Lys Val His Val His Ala Asp Gln Gly
1665                1670                1675                1680
Asp Gly Arg His Asp Asp Ala Asp Glu Arg Asp Ile Asp Pro His Val
                1685                1690                1695
Pro Val Asp His Ala Val Gly Glu Leu Ala Arg Ala Ala Cys His His
            1700                1705                1710
Val Ile Gly Leu Arg Val Asp Thr His Arg Leu Lys Ala Ser Gly Phe
        1715                1720                1725
Gln Ile Pro Ala Asp Asp Met Ala Glu Ile Asp Arg Ile Thr Gly Phe
    1730                1735                1740
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His Arg Phe Glu Arg His Val Gly